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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/869.098	09/20/2001	Yukio Toyoda	46342/56000	9857

7590 06/20/2006

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EXAMINER	
SCHLAPKOHL, WALTER	

ART UNIT	PAPER NUMBER
1636	

DATE MAILED: 06/20/2006

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/869,098	TOYODA ET AL.	
	Examiner	Art Unit	
	Walter Schlapkohl	1636	maf

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *27 March 2006*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1,5-8,11 and 17* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1,6,8,11 and 17* is/are rejected.

7)☒ Claim(s) *5,7 and 11* is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☒ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☒ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

Receipt is acknowledged of the papers filed 3/27/2006 in which claims 1, 6-8 and 11 were amended and claims 2-4 were cancelled. Claims 1, 5-8, 11 and 17 are pending and under examination in the instant Office Action.

Any rejection made in the previous Office Action not recited herein has been withdrawn.

Claim Objections

Claim 11 is objected to because of the following informalities: Claim 11 recites the phrase "wherein the part of the base sequence consists of nucleotides 255 to 430 of EQ ID NO: 1" in lines 9-23, and should instead read "wherein the part of the base sequence consists of nucleotides 255 to 430 of [[EQ]] SEQ ID NO: 1." Appropriate correction is required.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 6, 8, 11 and 17 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. **This a new rejection not necessitated by Applicant's amendment.**

Claim 6 recites "[t]he recombinant vector described in claim 5, which comprises a DNA comprising a structural gene under control of the human UCP-2 promoter region" in lines 1-3. Claim 6 is vague and indefinite in that it is unclear how a structural gene, which includes intron and exon sequences, as well as 5' regulatory regions and 3' untranslated regions, can be under the control of a human UCP-2 promoter. Which components of the gene are under the control of the human UCP-2 promoter? Does Applicant intend that the DNA of a coding region of a structural gene is under control of the human UCP-2 promoter or does Applicant intend that the whole structural gene is under the control of the human UCP-2 promoter region?

Claim 8 recites the phrase "[a] method for screening for a compound or its salt that promotes or inhibits a human UCP-2 promoter activity, which comprises: a. measuring the expression level of structural gene in a transformant with a human UCP-2 promoter sequence" in lines 1-4. Claim 8 is vague and indefinite in that it is unclear how the expression level of

structural gene is measured. Does Applicant intend such a method wherein the expression level of a reporter molecule is measured or does Applicant intend that the gene itself is measured?

Claim 11 recites the phrase "a structural gene inserted downstream of the human UCP-2 promoter" in line 29. Claim 11 is vague and indefinite in that it is unclear whether Applicant intends an entire structural gene, including intron and exon sequences, as well as 5' regulatory regions and 3' untranslated regions, located downstream of the human UCP-2 promoter, or whether Applicant intends a reporter molecule operatively linked to a human UCP-2 promoter region?

Claim 17 recites "[t]he kit of clima 11, wherein the structural gene is a luciferase gene" in lines 1-2. Claim 17 is also vague and indefinite for reasons as explained for claim 11, above.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

A person shall be entitled to a patent unless -

(b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Claim 1 is rejected under 35 U.S.C. 102(b) as being anticipated by Lewin (Genes V, Oxford University Press and Cell Press, pages 767-796, copyright 1994) as evidenced by GenBank entry Accession No. NM_003355. **This is a new rejection necessitated by Applicant's amendment.**

Lewin teaches an isolated DNA comprising a human uncoupling protein-2 (UCP-2) promoter region which consists of all or a part of a base sequence consisting of nucleotides 1-2270 of SEQ ID NO: 1, wherein the part of the base sequence consists of nucleotides 255 to 430 of SEQ ID NO: 1, nucleotides 255 to 717 of SEQ ID NO: 1, nucleotides 717 to 1133 of SEQ ID NO: 1, nucleotides 1133 to 1389 of SEQ ID NO: 1, nucleotides 255-1857 of SEQ ID NO: 1, nucleotides 571-2270 of SEQ ID NO: 1, nucleotides 717 to 2270 of SEQ ID NO: 1, nucleotides 1133 to 2270 of SEQ ID NO: 1, nucleotides 1389 to 2270 of SEQ ID NO: 1, or nucleotides 1634 to 2270 of SEQ ID NO: 1 (see especially page 781, second column and Figure 27.11). Isolated chromosome 11 is a DNA comprising a human UCP-2 promoter region consisting of all or part of the base sequence consisting of nucleotides 1-2270 of SEQ ID NO: 1, wherein the part of the base sequence is each of the parts recited in the claim. The UCP-2 promoter region is

present on chromosome 11 as evidenced by GenBank entry Accession No. NM_003355.

Allowable Subject Matter

Claims 5 and 7 are objected to as being dependent upon a rejected base claim, but would be allowable if rewritten in independent form including all of the limitations of the base claim and any intervening claims. Claims 5 and 7 are drawn to a recombinant vector comprising the DNA described in claim 1 (claim 5) and a transfomant transformed with such a vector (claim 7). Lewin teaches an isolated DNA comprising a human uncoupling protein-2 (UCP-2) promoter region, which consists of all or a part of a base sequence consisting of nucleotides 1-2270 of SEQ ID NO: 1, wherein the part of the base sequence consists of nucleotides 255 to 430 of SEQ ID NO: 1, nucleotides 255 to 717 of SEQ ID NO: 1, nucleotides 717 to 1133 of SEQ ID NO: 1, nucleotides 1133 to 1389 of SEQ ID NO: 1, nucleotides 255-1857 of SEQ ID NO: 1, nucleotides 571-2270 of SEQ ID NO: 1, nucleotides 717 to 2270 of SEQ ID NO: 1, nucleotides 1133 to 2270 of SEQ ID NO: 1, nucleotides 1389 to 2270 of SEQ ID NO: 1, or nucleotides 1634 to 2270 of SEQ ID NO: 1, but Lewin does not teach such an isolated DNA wherein the DNA is a recombinant vector.

Conclusion

Certain papers related to this application may be submitted to the Art Unit 1636 by facsimile transmission. The faxing of such papers must conform with the notices published in the Official Gazette, 1156 OG 61 (November 16, 1993) and 1157 OG 94 (December 28, 1993) (see 37 C.F.R. § 1.6(d)). The official fax telephone number for the Group is (571) 273-8300. Note: If Applicant *does* submit a paper by fax, the original signed copy should be retained by Applicant or Applicant's representative. NO DUPLICATE COPIES SHOULD BE SUBMITTED so as to avoid the processing of duplicate papers in the Office.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to (571) 272-0547.

Patent applicants with problems or questions regarding electronic images that can be viewed in the Patent Application Information Retrieval system (PAIR) can now contact the USPTO's Patent Electronic Business Center (Patent EBC) for assistance. Representatives are available to answer your questions daily from 6 am to midnight (EST). The toll free number is (866) 217-9197. When calling please have your application serial or patent number, the type of document you are having an image problem with, the number of pages and the specific nature of the problem. The Patent Electronic Business Center will notify applicants of the resolution of the problem within 5-7 business days. Applicants can also check PAIR to confirm that the problem has been corrected. The USPTO's Patent Electronic Business Center is a complete service center supporting all patent business on the Internet. The USPTO's PAIR system provides Internet-based access to patent applications to view

the scanned images of their own application file folder(s) as well as general patent information available to the public.

For all other customer support, please call the USPTO Call Center (UCC) at (800) 786-9199.

Any inquiry concerning rejections or objections in this communication or earlier communications from the examiner should be directed to Walter Schlapkohl whose telephone number is (571) 272-4439. The examiner can normally be reached on Monday through Thursday from 8:30 AM to 6:00 PM. The examiner can also be reached on alternate Fridays.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Dr. Remy Yucel can be reached at (571) 272-0781.

Walter A. Schlapkohl, Ph.D.
Patent Examiner
Art Unit 1636

June 8, 2006

NANCY VOGEL
PRIMARY EXAMINER

NANCY VOGEL
PRIMARY EXAMINER

Notice of References Cited	Application/Control No. 09/869,098	Applicant(s)/Patent Under Reexamination TOYODA ET AL.	
	Examiner Walter Schlapkohl	Art Unit 1636	Page 1 of 1

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	Classification
	A	US-			
	B	US-			
	C	US-			
	D	US-			
	E	US-			
	F	US-			
	G	US-			
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	I	US-			
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FOREIGN PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Country	Name	Classification
	N					
	O					
	P					
	Q					
	R					
	S					
	T					

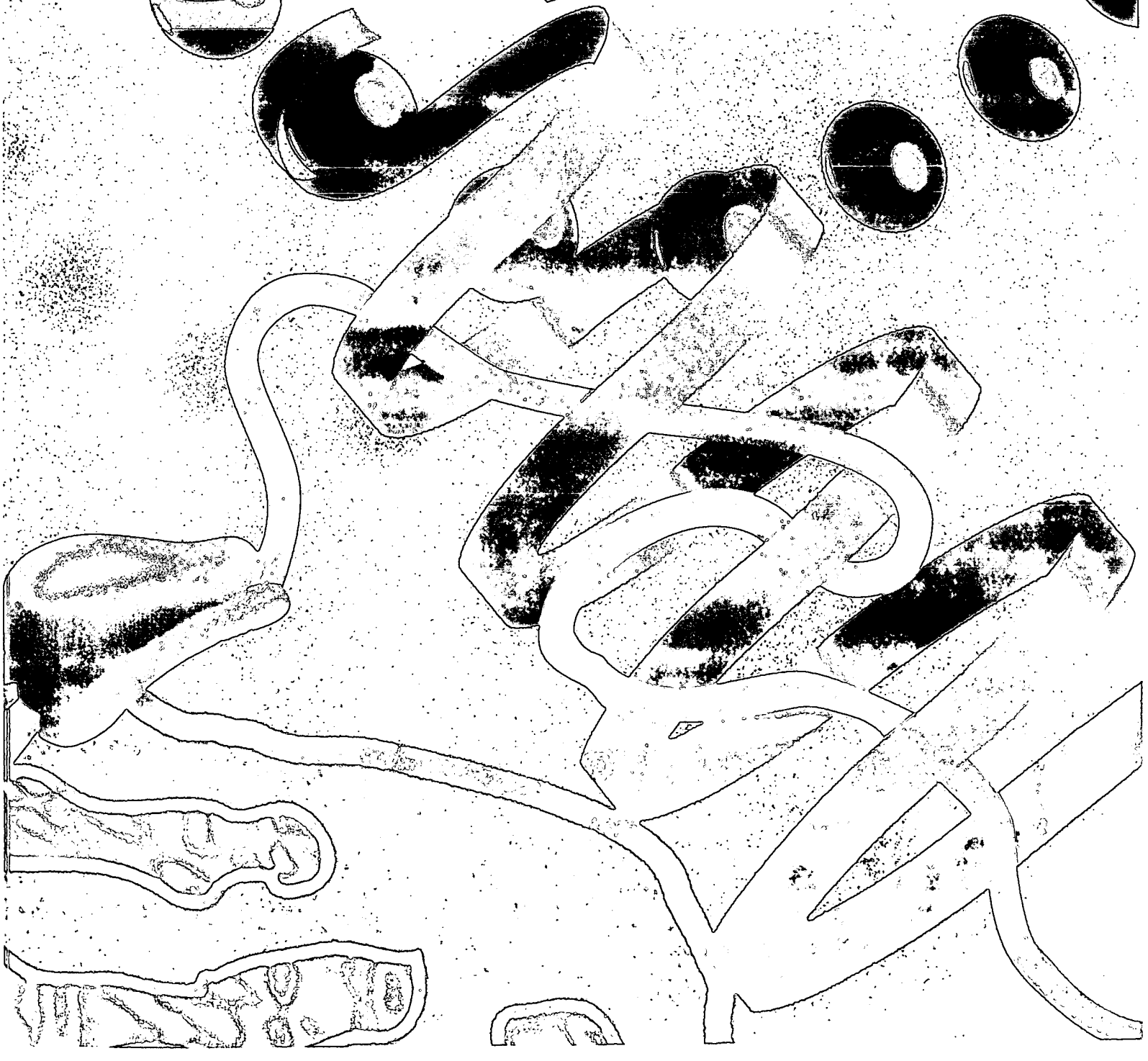
NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	Lewin, Genes V, Oxford University Press and Cell Press, pages 767-796, copyright 1994.
	V	GenBank Accession No. NM_003355
	W	
	X	

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

BENJAMIN LEWIN

GENES V



Oxford University Press, Walton Street, Oxford OX2 6DP

Oxford New York
Athens Auckland Bangkok Bombay
Calcutta Cape Town Dar es Salaam Delhi
Florence Hong Kong Istanbul Karachi
Kuala Lumpur Madras Madrid Melbourne
Mexico City Nairobi Paris Singapore
Taipei Tokyo Toronto

Oxford is a trade mark of Oxford University Press

Published in the United States
by Oxford University Press Inc., New York

First published 1994
Reprinted with corrections 1994 (twice)

A catalogue record for this book is available from the British Library

Library of Congress Cataloging in Publication Data

Printed in U.S.A.

CHAPTER 27

The genome is packaged into chromosomes

A general principle is evident in the organization of all cellular genetic material. It exists as a compact mass, occupying a limited volume; and its various activities, such as replication and transcription, must be accomplished within these confines. The organization of this material must accommodate transitions between inactive and active states.

The condensed state of nucleic acid results from its binding to basic proteins. The positive charges of these proteins neutralize the negative charges of the nucleic acid. The structure of the nucleoprotein complex is determined by the interactions of the proteins with the DNA (or RNA).

A common problem is presented by the packaging of DNA into phages and viruses, into bacterial cells and eukaryotic nuclei. The length of the DNA as an extended molecule would vastly exceed the dimensions of the compartment that contains it. The DNA (or in the case of some viruses, the RNA) must be compressed exceedingly tightly to fit into the space available. *Thus in contrast with the customary picture of DNA as an extended double helix, structural deformation of DNA to bend or fold it into a more compact form is the rule rather than exception.*

The magnitude of the discrepancy between the length of the nucleic acid and the size of its compartment is evident from the examples summarized in **Table 27.1**. For bacteriophages and for eukaryotic viruses, whether rod-like or spherical, the nucleic acid genome, whether DNA or RNA, whether single-stranded or double-stranded, effectively fills the container.

For bacteria or for eukaryotic cell compartments, the discrepancy is hard to calculate exactly, because the DNA is contained in a compact area that occupies only part of the compartment. The genetic material is seen in the form of the **nucleoid** in bacteria and as the mass of **chromatin** in eukaryotic nuclei at interphase (between divisions).

The density of DNA in these compartments is high. In a bacterium it is ~10 mg/ml, in a eukaryotic nucleus it is ~100 mg/ml, and in the phage T4 head it is >500 mg/ml. Such a concentration in solution would be equivalent to a gel of great viscosity. We do not entirely understand the physiological implications, for example, what effect this has upon the ability of proteins to find their binding sites on DNA.

The packaging of chromatin is flexible; it changes during the eukaryotic cell cycle. At the time of division (mitosis or meiosis), the genetic material becomes even more tightly packaged, and individual **chromosomes** become recognizable.

The overall compression of the DNA can be described by the **packing ratio**, the length of the DNA divided by the length of the unit that contains it. For example, the smallest human chromosome contains ~4.6 × 10^7 bp of DNA (~10 times the genome size of the bacterium *E. coli*). This is equivalent to 14,000 μm (= 1.4 cm) of extended DNA. At the most condensed moment of mitosis, the

Table 27.1

The length of nucleic acid is much greater than the dimensions of the surrounding compartment.

Compartment	Shape	Dimensions	Type of Nucleic Acid	Length		
TMV	Filament	0.008 x 0.3 μm	1 single-stranded RNA	2μm	=	6.4 kb
Phage fd	Filament	0.006 x 0.85 μm	1 single-stranded DNA	2μm	=	6 kb
Adenovirus	Icosahedron	0.07μm diameter	1 double-stranded DNA	11μm	=	35 kb
Phage T4	Icosahedron	0.065 x 0.10 μm	1 double-stranded DNA	55μm	=	170 kb
E. coli	Cylinder	1.7 x 0.65 μm	1 double-stranded DNA	1.3mm	=	4.2×10^3 kb
Mitochondrion (human)	Oblate Spheroid	3.0 x 0.5 μm	~10 identical double-stranded DNAs	50μm	=	16 kb
Nucleus (human)	Spheroid	6μm diameter	46 chromosomes of double-stranded DNA	1.8m	=	6×10^6 kb

chromosome is ~2 μm long. Thus the packing ratio of DNA in the chromosome can be as great as 7000.

Packing ratios cannot be established with such certitude for the more amorphous overall structures of the bacterial nucleoid or eukaryotic chromatin. However, the usual reckoning is that mitotic chromosomes are likely to be 5–10 times more tightly packaged than interphase chromatin, which therefore has a typical packing ratio of 1000–2000.

A major unanswered question concerns the *specificity* of packaging. Is the DNA folded into a *particular* pattern, or is it different in each individual copy of the genome? How does the pattern of packaging change when a segment of DNA is replicated or transcribed?

Condensing viral genomes into their coats

From the perspective of packaging the *individual* sequence, there is an important difference between a cellular genome and a virus. The cellular genome is essentially indefinite in size; the number and location of individual sequences can be changed by duplication, deletion, and rearrangement. Thus it requires a *generalized* method for packaging its DNA, insensitive to the total content or distribution of sequences. By contrast, two restrictions define the needs of a virus. The amount of nucleic acid to be packaged is *predetermined* by the size of the genome. And it must all fit within a coat assembled from a protein or proteins coded by the viral genes.

A virus particle is deceptively simple in its superficial appearance. The nucleic acid genome is contained within a capsid, a symmetrical or quasi-symmetrical structure assembled from one or only a few proteins. Attached to the capsid or incorporated into it, are other structures, assembled from distinct proteins, and necessary for infection of the host cell.

The virus particle is tightly constructed. The

...nal volume of the capsid is rarely much greater ...n the volume of the nucleic acid it must hold. The difference is usually less than twofold, and often the internal volume is barely larger than the nucleic acid.

In its most extreme form, the restriction that the capsid must be assembled from proteins coded by the virus means that the entire shell is constructed from a single type of subunit. The rules for assembly of identical subunits into closed structures restrict the capsid to one of two types. The protein subunits stack sequentially in a helical array to form a **filamentous** or rodlike shape. Or they form a pseudospherical shell, a type of structure that conforms to a polyhedron with **icosahedral symmetry**. Some viral capsids are assembled from more than a single type of protein subunit, but although this extends the exact types of structures that can be formed, viral capsids still all conform to the general classes of quasi-crystalline filaments or icosahedrons.

There are two types of solution to the problem of how to construct a capsid that contains nucleic acid:

- The protein shell can be assembled around the nucleic acid, condensing the DNA or RNA by protein–nucleic acid interactions during the process of assembly.
- Or the capsid can be constructed from its component(s) in the form of an empty shell, into which the nucleic acid must be inserted, being condensed as it enters.

Assembly of the capsid around the genome occurs in the case of single-stranded RNA viruses. The best characterized example is TMV (tobacco mosaic virus). Assembly starts at a duplex hairpin that lies within the RNA sequence. From this **nucleation center**, it proceeds bidirectionally along the RNA, until reaching the ends. The unit of the capsid is a two-layer disk, each layer containing 17 identical protein subunits. The disk is a circular structure, which forms a helix as it interacts with the RNA. The RNA becomes coiled in a helical array on the inside of the protein shell, as illustrated in **Figure 27.1**.

The structure of the RNA genome is determined by its interaction with the protein shell. An analogous arrangement is presented by spherical capsids that contain single-stranded RNA, for example, TYMV (turnip yellow mosaic virus). The common principle is that *the position of the RNA within the capsid is determined directly by its binding to the proteins of the shell.*

The spherical capsids of DNA viruses are assembled in a different way, as best characterized for the phages lambda and T4. In each case, an empty head shell is assembled from a small set of proteins. *Then the duplex genome is inserted into the head,* a process accompanied by a structural change in the capsid.

Figure 27.2 summarizes the assembly of lambda. It starts with a small head shell that contains a

Figure 27.1

A helical path for TMV RNA is created by the stacking of protein subunits in the virion.

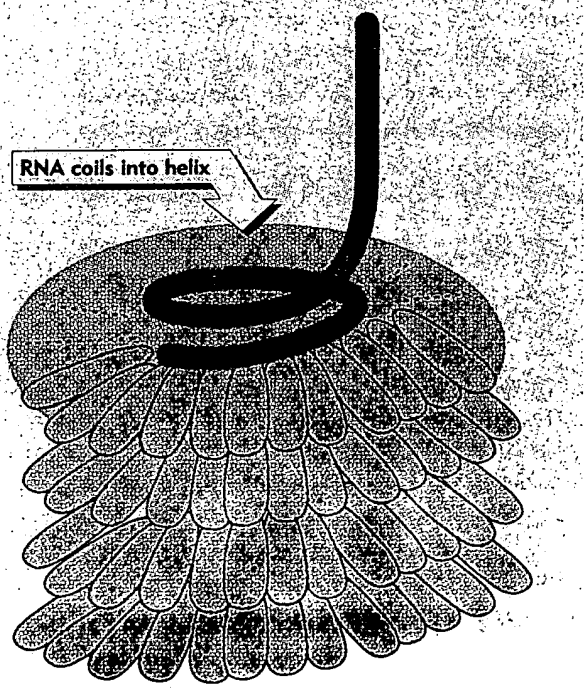
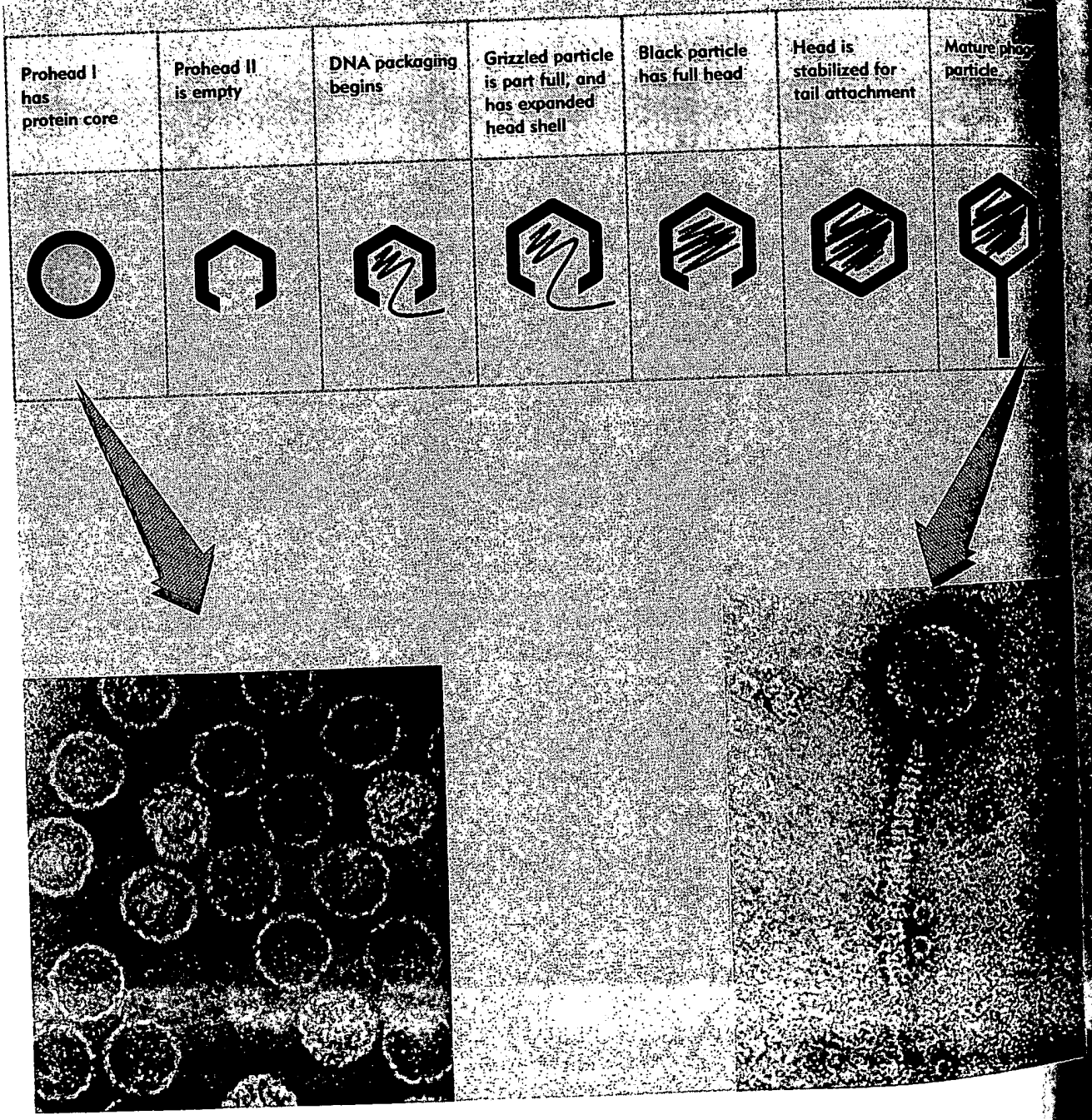


Figure 27.2

Maturation of phage lambda passes through several stages. The empty head changes shape and expands when it becomes filled with DNA. The electron micrographs show the particles at the start and end of the maturation pathway. Photographs kindly provided by A. F. Howatson.



protein 'core'. This is converted to an empty head shell of more distinct shape. Then DNA packaging begins, the head shell expands in size though remaining the same shape, and finally the full head is sealed by addition of the tail.

For both lambda and T4, the DNA that is to be inserted into the empty head takes the form of **concatemeric** molecules. These are multiple genomes joined end to end, as depicted in Figure 27.3. Each phage has its own mechanism for recognizing the proper amount of DNA to insert. The ends of the lambda genome are marked by sequences called *cos* sites. Cleavage occurs at the left *cos* site (as defined on the usual map) to generate a free end that is inserted into the capsid. The insertion of DNA continues until the right *cos* site is encountered, when it is cleaved to generate the other end. The end that goes into the capsid last during assembly comes out first when a new host cell is infected.

Any DNA contained between two *cos* sites can be packaged. (This is the basis of the 'cosmid' cloning technique described in Chapter 21.) Although the sequence of DNA is irrelevant, its length is important: the distance between the *cos* sites can be varied only slightly from the usual length of lambda DNA. Packaging does not occur if the distance is either too great or too small. This demonstrates that there must be *enough* DNA to complete the packaging reaction, as well as showing that there is room in the head only for a very little extra DNA (~15%).

With phage T4, insertion starts at a *random* point in the concatemeric precursor. It continues until a genome's worth of DNA (a 'headful') has been inserted. This implies the existence of some mechanism for measuring the amount of DNA. (Actually, the amount inserted is slightly greater than the length of the unit genome, creating a **terminal redundancy** corresponding to the additional length. In the terms of Figure 27.3, the first virion might contain the DNA from *A* to *A*, the next from *B* to *B*, and so on, so that each genome has a [different] letter repeated at each end.)

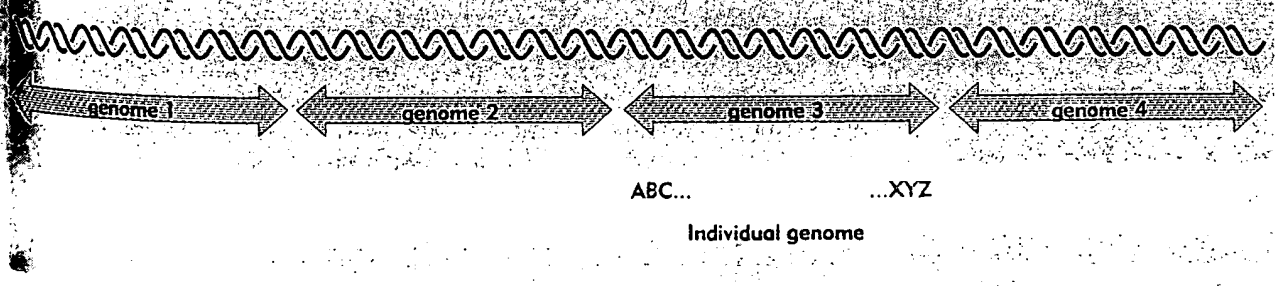
Now a double-stranded DNA considered over short distances is a fairly rigid rod. Yet it must be compressed into a compact structure to fit within the capsid. We should like to know whether packaging involves a smooth coiling of the DNA into the head or requires abrupt bends.

Inserting DNA into a phage head involves two types of reaction: translocation and condensation. Both are energetically unfavorable.

Translocation appears to be an active process in

Figure 27.3

Concatemeric DNA consists of a tandem series of phage genomes.



which the DNA is driven into the head by an ATP-dependent mechanism. One possibility is that the terminase enzymes that generate the ends of DNA could be involved in pushing it into the head. Another possibility is that the capsid protein(s) could pull the DNA in.

Little is known about the mechanism of condensation, except that the capsid contains 'internal proteins' as well as DNA. One possibility is that they provide some sort of 'scaffolding' onto which the DNA condenses. (This would be a counterpart to the use of the proteins of the shell in the plant RNA viruses.)

How specific is the packaging? It cannot depend on particular sequences, because deletions, insertions, and substitutions all fail to interfere with the assembly process. The relationship between DNA and the head shell has been investigated directly by determining which regions of the DNA can be chemically cross-linked to the proteins of the capsid. The surprising answer is that all regions of the DNA are more or less equally susceptible. This probably means that when DNA is inserted into the head, it follows a general rule for condensing, but the pattern is not determined by particular sequences.

These varying mechanisms of virus assembly all accomplish the same end: packaging a single DNA or RNA molecule into the capsid. However, some viruses have genomes that consist of multiple nucleic acid molecules. Reovirus contains ten double-stranded RNA segments, all of which must be packaged into the capsid. Specific sorting sequences in the segments may be requ... ensure that the assembly process selects o... of each different molecule in order to col... complete set of genetic information.

Some plant viruses are multipartite: the... genomes consist of segments each of which is pac... aged into a *different* capsid. An example is alfa... mosaic virus, which has four different ... stranded RNAs, each packaged independen... a coat comprising the same protein subuni... cessful infection depends on the entry of one ... type into the cell.

The four components of the virus ... particles of different sizes. This means th... same capsid protein can package each RN... own characteristic particle. This is a deg... from the packaging of a unique length of ... acid into a capsid of fixed shape.

The assembly pathway of viruses whose ... have only one authentic form may be dive... mutations that cause the formation of ab... monster particles in which the head is longe... usual. These mutations show that a ca... protein(s) has an intrinsic ability to assemb... a particular type of structure, but the exact di... shape may vary. Some of the mutations oc... genes that code for assembly proteins, whic... needed for head formation, but are not them... part of the head shell. Such ancillary protein... the options of the capsid protein so that it asse... only along the desired pathway. Comp... proteins are employed in the assembly of c... chromatin (see Chapter 28).

The bacterial genome is a nucleoid with many supercoiled loops

Although bacteria do not display structures with the distinct morphological features of eukaryotic chromosomes, their genomes nonetheless are organized into definite bodies. The genetic material can be seen as a fairly compact clump or series of clumps that occupies about a third of the volum... the cell. **Figure 27.4** displays a thin section thr... bacterium in which this nucleoid is eviden...

In bacteria that have partially replicat... DNA, the nucleoid contains more than ...

Figure 27.4

A thin section shows the bacterial nucleoid as a compact mass in the center of the cell. Photograph kindly provided by Jack Griffith.

genome's worth of DNA. By the time of cell division, the material has separated into two nucleoids that are partitioned into the daughter cells. A bacterium does not organize a spindle like a eukaryotic cell; the segregation mechanism probably involves attachment of the bacterial genome to the envelope, allowing segregation to occur by active movement or passive separation (see Chapter 18). At all events, when division occurs each nucleoid is in a different compartment.

When *E. coli* cells are lysed, fibers are released in the form of loops attached to the broken envelope of the cell. As can be seen from **Figure 27.5**, the DNA of these loops is not found in the extended form of a free duplex, but is compacted by association with proteins.

Several DNA-binding proteins with a superficial resemblance to eukaryotic chromosomal proteins have been isolated in *E. coli*. What criteria should we apply for deciding whether a DNA-binding protein plays a structural role in the nucleoid? It should be present in sufficient quantities to bind throughout the genome. And mutations in its gene should cause some disruption of structure or of functions associated with genome survival (for example, segregation to daughter cells). None of the candidate proteins yet satisfies the genetic conditions.

Protein HU is a dimer that condenses DNA, possibly wrapping it into a bead-like structure. It stimulates DNA replication (see Chapter 19). It is related to IHF (integration host factor), another dimer, which is involved in some specialized recombination reactions, including the integration and excision of phage lambda (for which it is named), and has a structural role in building a protein complex that holds reacting DNA sequences in apposition (see Chapter 33). Null mutations in either of the genes coding for the subunits of HU (*hupA,B*) have little effect, but loss of both functions causes a cold-sensitive phenotype and some loss of superhelicity in DNA. These results raise the possibility that HU plays a role in nucleoid condensation that is a non-sequence specific counterpart to the specific role played by IHF in lambda recombination.

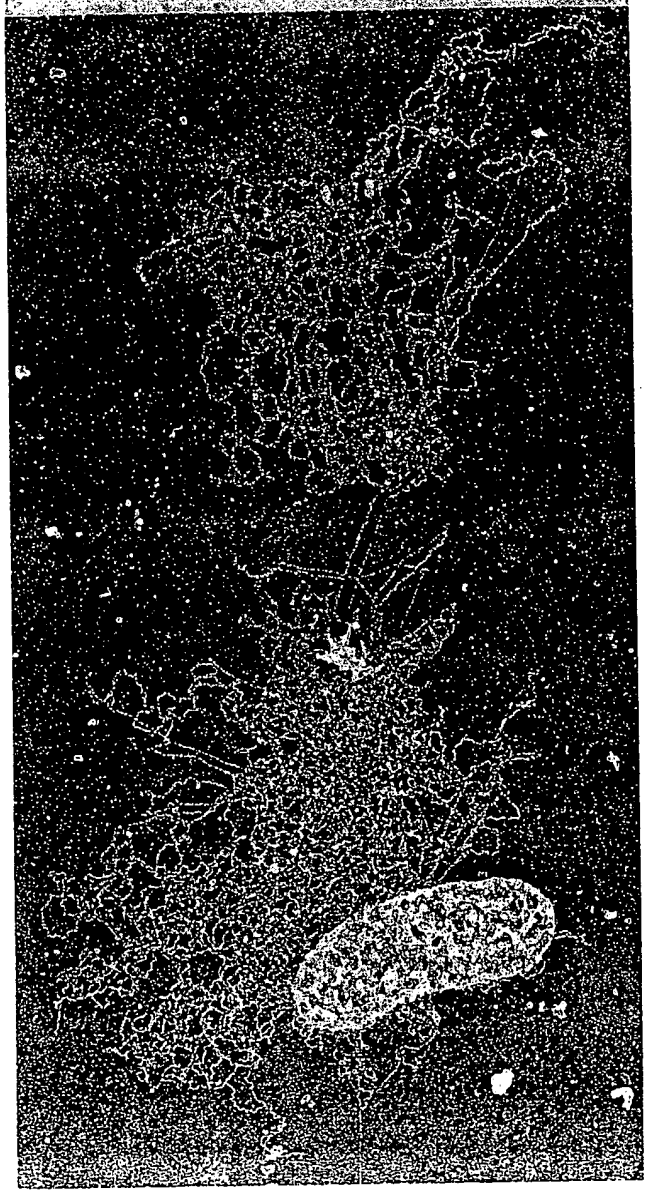
Protein H1 (also known as H-NS) binds DNA, interacting preferentially with sequences that are bent. Mutations in its gene have turned up in a variety of guises (*osmZ, bglY, pilG*), each identified as an apparent regulator of a different system. These results probably reflect the effect that H1 has on the local topology of DNA, with effects upon gene expression that depend upon the particular promoter.

Protein P has been identified by sequencing a gene whose coding region has an amino acid composition resembling the protamines that bind to DNA in certain sperm. Its sequence suggests it is a DNA-binding protein, but its quantity and functions are unknown.

We might expect that the absence of a protein required for nucleoid structure would have serious effects upon viability. Why then are the effects of deletions in the genes for proteins HU and H1 only relatively restricted? One explanation is that these proteins are *redundant*, that any one can substitute for the others, so that deletions of *all* of them would

Figure 27.5

The nucleoid spills out of a lysed *E. coli* cell in the form of loops of a fiber. Photograph kindly provided by Jack Griffith.



be necessary to interfere seriously with nucleoid structure. Another possibility is that we have yet to identify the proteins responsible for the major features of nucleoid integrity.

The nucleoid can be isolated directly in the form of a very rapidly sedimenting complex, consisting of ~80% DNA by mass. (The analogous complexes in eukaryotes have ~50% DNA by mass; see later.) It can be unfolded by treatment with reagents that act on RNA or protein. The possible role of proteins in stabilizing its structure is evident. The role of RNA has been quite refractory to analysis, and we do not understand it.

The DNA of the compact body isolated *in vitro* behaves as a closed duplex structure, as judged by its response to ethidium bromide. This small molecule intercalates between base pairs to generate *positive* superhelical turns in 'closed' circular DNA molecules, that is, molecules in which both strands have covalent integrity. (In 'open' circular molecules, which contain a nick in one strand, or with linear molecules, the DNA can rotate freely in response to the intercalation, thus relieving the tension.)

In a natural closed DNA that is *negatively* supercoiled, the intercalation of ethidium bromide first removes the negative supercoils and then introduces positive supercoils. The amount of ethidium bromide needed to achieve zero supercoiling is a measure of the original density of negative supercoils.

Some nicks occur in the compact nucleoid during its isolation; they can also be generated by limited treatment with DNAase. But this does not abolish the ability of ethidium bromide to introduce positive supercoils. This capacity of the genome to retain its response to ethidium bromide in the face of nicking means that it must have many independent domains; the supercoiling in each domain is not affected by events in the other domains.

This autonomy suggests that the structure of the bacterial chromosome has the general organization depicted diagrammatically in Figure 27.6. Each domain consists of a loop of DNA, the ends of which are secured in some (unknown) way that does not allow rotational events to propagate from one domain to another. There are ~100 such domains per genome; each consists of ~40 kb (15 μm) of DNA, organized into some more compact fiber whose structure has yet to be characterized.

The existence of separate domains could permit

degrees of supercoiling to be maintained rent regions of the genome. This is a per- factor in considering the different ibilities of particular bacterial promoters to percoiling (see Chapter 14).

Supercoiling in the genome can in principle take orms:

upercoiled DNA is free, its path is **un-trained**, and negative supercoils generate a e of torsional tension that is transmitted freely ng the DNA within a domain. It can be relieved unwinding the double helix, as described in pter 5. The DNA is in a dynamic equilibrium ween the states of tension and unwinding.

ercoiling can be **restrained** if proteins are nd to the DNA to hold it in a particular three- imensional configuration. In this case, the ercoils are represented by the path the DNA lows in its fixed association with the proteins. e energy of interaction between the proteins d the supercoiled DNA stabilizes the nucleic ci so that no tension is transmitted along the olecule.

re the supercoils in *E. coli* DNA restrained or is the double helix subject to the torsional on characteristic of free DNA? Measurements percoiling *in vitro* encounter the difficulty that raining proteins may have been lost during tion. Various approaches suggest that DNA is r torsional stress *in vivo*, although it is difficult quantitate the level of supercoiling.

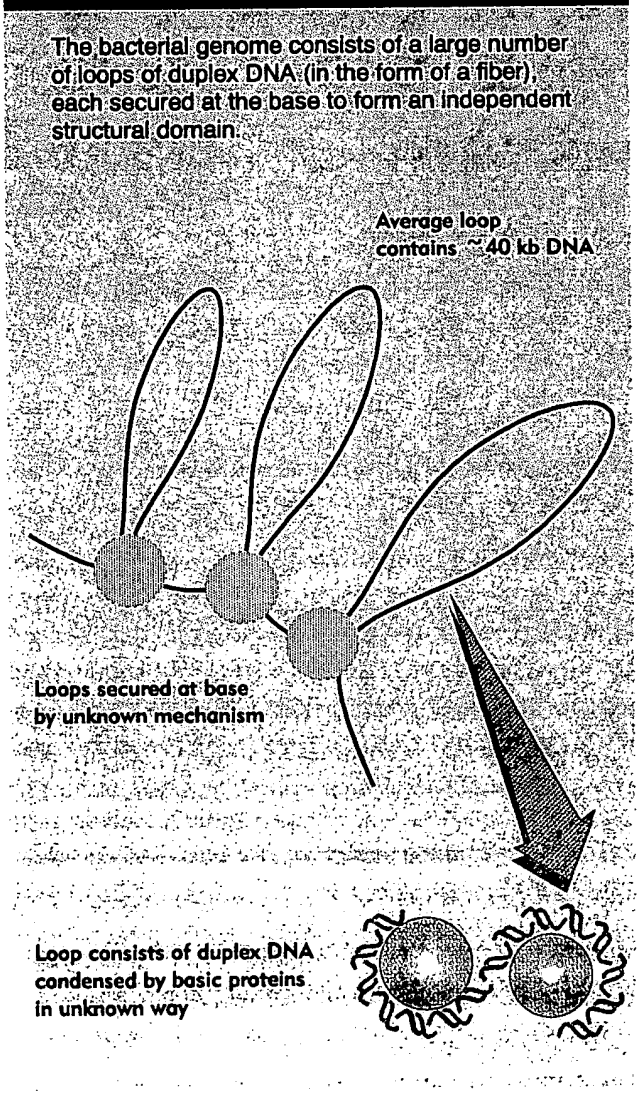
direct approach is to use the cross-linking ent psoralen, which binds more readily to DNA it is under torsional tension. The reaction of alen with *E. coli* DNA *in vivo* corresponds to an erage density of one negative superhelical turn every 200 bp ($\sigma = -0.05$).

Another approach is to examine the ability cells to form alternative DNA structures; for ample, to generate cruciforms at palindromic equences. From the change in linking number is required to drive such reactions, it is ible to calculate the original supercoiling density. This approach suggests an average density of $\sigma = -0.025$, or one negative superhelical turn per 100 bp.

These results therefore demonstrate that supercoils *do* create torsional tension *in vivo*. There may be variation about an average level, and although the precise range of densities is difficult to measure, it is clear that the level is sufficient to exert significant effects on DNA structure, for example, in assisting melting in particular regions such as origins or promoters.

Many of the important features of the structure

Figure 27.6



of the compact nucleoid remain to be established. What is the specificity with which domains are constructed—do the same sequences always lie at the same relative locations, or can the contents of individual domains shift? How is the integrity of the domain maintained? Biochemical analysis by itself is unable to answer these questions fully, but if it is possible to devise suitable selective techniques, the properties of structural mutants should lead to a molecular analysis of nucleoid construction.

Loops, domains, and scaffolds in eukaryotic DNA

Interphase chromatin appears to be a tangled mass occupying a large part of the nuclear volume, in contrast with the highly organized and reproducible ultrastructure of mitotic chromosomes. What controls the distribution of interphase chromatin within the nucleus?

Some indirect evidence on its nature is provided by the isolation of the genome as a single, compact body. Using the same technique just described for isolating the bacterial nucleoid, nuclei can be lysed on top of a sucrose gradient. This releases the genome in a form that can be collected by centrifugation. As isolated from *D. melanogaster*, it can be visualized as a compactly folded fiber (10 nm in diameter), consisting of DNA bound to proteins.

Supercoiling measured by the response to ethidium bromide corresponds to about one negative supercoil for every 200 bp. These supercoils can be removed by nicking with DNAase, although the DNA remains in the form of the 10 nm fiber. This suggests that the supercoiling is caused by the arrangement of the fiber in space, and represents the existing torsion.

Full relaxation of the supercoils requires one nick for every 85 kb. Thus the average length of 'closed' DNA is ~85 kb. This region could comprise a loop or domain similar in nature to those identified in the bacterial genome. We should like to know whether these loops correspond to specific sequences and whether they have functional significance.

Loops can be seen directly when the majority of proteins are extracted from mitotic chromosomes. The resulting complex consists of the DNA associated with ~8% of the original protein content. As seen in **Figure 27.7**, the protein-depleted chromosomes take the form of a central scaffold surrounded by a halo of DNA.

The metaphase scaffold consists of a dense network of fibers. Threads of DNA emanate from the scaffold, apparently as loops of average length 10–30 μm (30–90 kb). The DNA can be digested without affecting the integrity of the scaffold, which consists of a set of specific proteins. This suggests a form of organization in which loops of DNA of ~60 kb are anchored in a central proteinaceous scaffold.

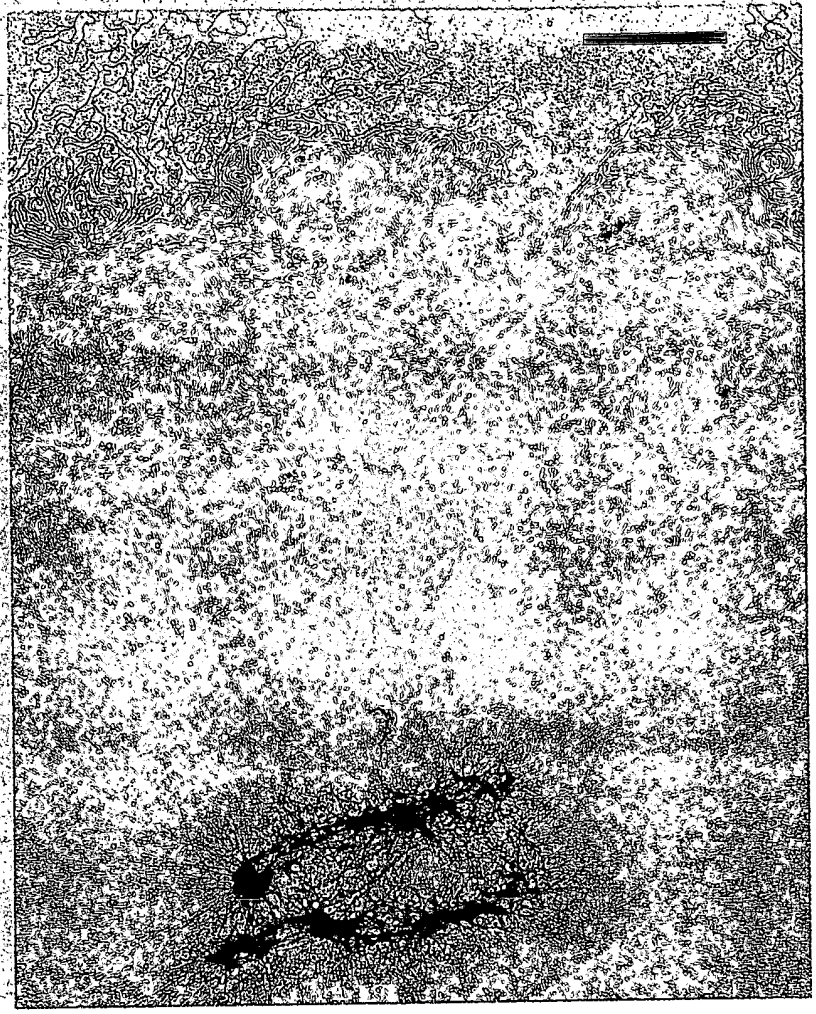
The appearance of the scaffold resembles a mitotic pair of sister chromatids. The sister scaffolds usually are tightly connected, but sometimes are separate, joined only by a few fibers. Could this be the structure responsible for maintaining the shape of the mitotic chromosomes? Could it be generated by bringing together the protein components that usually secure the bases of loops in interphase chromatin?

Interphase cells possess a *nuclear matrix*, a filamentous structure on the interior of the nuclear membrane. Chromatin often appears to be attached to the matrix, and there have been many suggestions that such attachment is necessary for transcription or replication. When nuclei are depleted of proteins, the DNA extrudes as loops from the residual nuclear matrix.

Is DNA attached to the matrix or scaffold via

Figure 27.7

Histone-depleted chromosomes consist of a protein scaffold to which loops of DNA are anchored. Photograph kindly provided by Ulrich K. Laemmli.



specific sequences? DNA sites attached to proteinaceous structures in interphase nuclei are called **MAR** (matrix attachment regions); it is confusing that they are sometimes also called **SAR** (scaffold attachment regions), although they concern the nuclear matrix.

How might we demonstrate that particular DNA regions are genuinely associated with the matrix? *In vivo* and *in vitro* approaches are summarized in **Figure 27.8**. Both start by isolating the matrix as a crude nuclear preparation containing chromatin and nuclear proteins. Different treatments can then be used to characterize DNA in the matrix or to identify DNA able to attach to it.

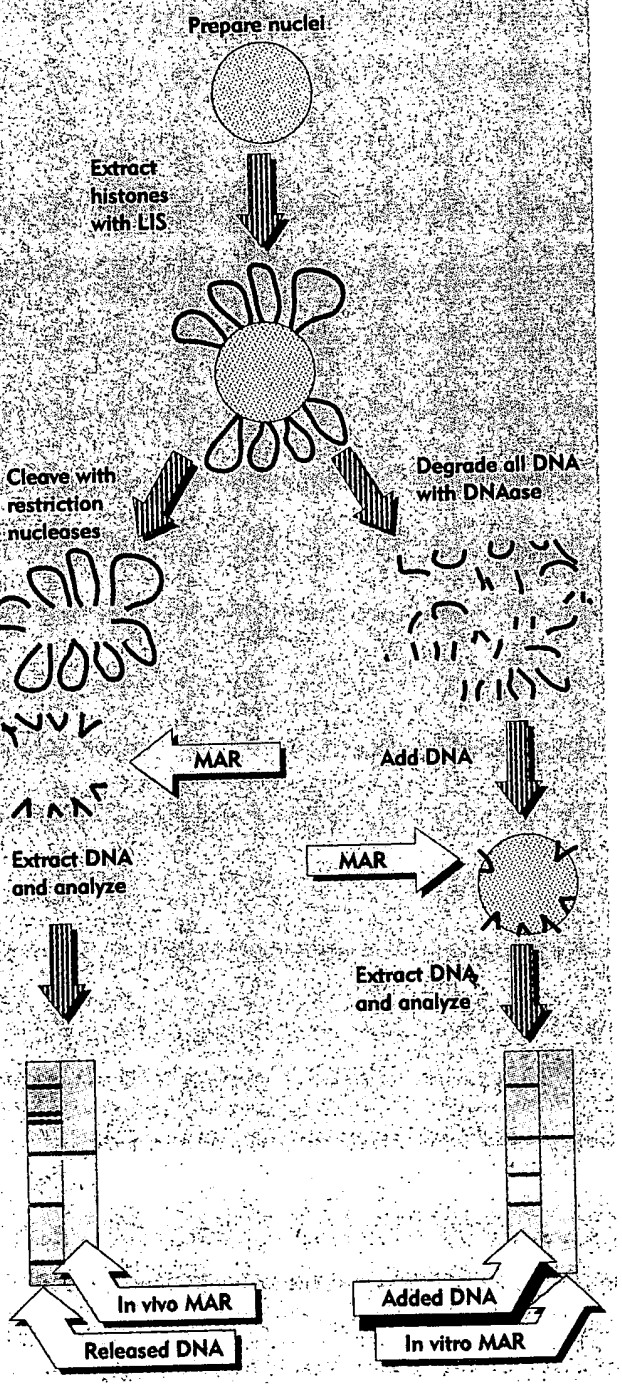
To analyze the existing MAR, the chromosomal loops can be decondensed by extracting the proteins. Removal of the DNA loops by treatment with restriction nucleases leaves only the (presumptive) *in vivo* MAR sequences attached to the matrix.

The complementary approach is to remove *all* the DNA from the matrix by treatment with DNAase; then isolated fragments of DNA can be tested for their ability to bind to the matrix *in vitro*.

The same sequences should be associated with the matrix *in vivo* or *in vitro*. Once a potential MAR

Figure 27.8

Matrix-associated regions may be identified by characterizing the DNA retained by the matrix isolated in vivo or by identifying the fragments that can bind to the matrix from which all DNA has been removed *in vivo*.



has been identified, the size of the minimal region needed for association *in vitro* can be determined by deletions. Point mutations of the MAR should prevent it from associating with the matrix. Specific matrix proteins should bind to the MAR, and it should in principle be possible to identify these proteins via their ability to recognize the specific DNA sequences of the MAR.

Several MAR sequences have been identified by the criteria of matrix binding *in vivo* or *in vitro*. We have not yet reached the stage of systematic mutation of the attachment sequences. Nor have MAR-binding proteins yet been characterized.

What is the relationship between the chromosome scaffold of dividing cells and the nuclear matrix of interphase cells; are the same DNA sequences attached to both structures? In several cases, the same DNA fragments that are found with the nuclear matrix *in vivo* can be retrieved from the metaphase scaffold. And fragments that contain MAR sequences can bind to a metaphase scaffold. It therefore seems likely that DNA contains a single type of attachment site, which in interphase cells is connected to the nuclear matrix, and in mitotic cells is connected to the chromosome scaffold.

The nuclear matrix and chromosome scaffold consist of different proteins, although there are some common components. In particular, topoisomerase II is a prominent component of the chromosome scaffold, and is a constituent of the nuclear matrix. We have yet to quantitate the proportion of the enzyme in the cell that is matrix- or scaffold-associated.

A surprising feature is the lack of conservation of sequence in MAR fragments. They are usually ~70% A•T-rich, but otherwise lack any consensus sequences. However, other interesting sequences often are in the DNA stretch containing the MAR. *Cis*-acting sites that regulate transcription are common. And a recognition site for topoisomerase II is usually present in the MAR. It is therefore possible that an MAR serves more than one function, providing a site for attachment to the matrix, but also containing other sites at which topological changes in DNA are effected.

The contrast between interphase chromatin and mitotic chromosomes

Individual eukaryotic chromosomes come into the limelight for only a brief period, during the act of cell division. Only then can each be seen as a compact unit. **Figure 27.9** is an electron micrograph of a sister chromatid pair, captured at metaphase. (The sister chromatids are daughter chromosomes produced by the previous replication event, still joined together at this stage of mitosis, as described in Chapter 2.) Each consists of a fiber with a diameter of ~30 nm and a nubbly appearance. The DNA is 5–10× more condensed in chromosomes than it is at interphase.

During most of the life cycle of the eukaryotic cell, however, its genetic material occupies an area of the nucleus in which individual chromosomes cannot be distinguished. The structure of the interphase chromatin does not change visibly between divisions. No disruption is evident during the period of replication, when the amount of chromatin doubles. Chromatin is fibrillar, although the overall configuration of the fiber in space is hard to discern in detail. The fiber itself, however, is similar or identical to that of the mitotic chromosomes.

Chromatin can be divided into two types of material, which can be seen in the nuclear section of Figure 27.10:

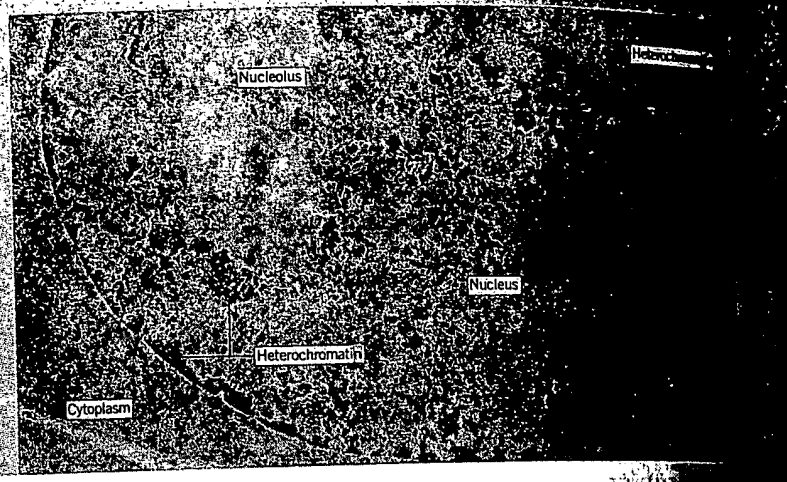
- In most regions, the fibers are much less densely packed than in the mitotic chromosome. This material is called **euchromatin**. It has a relatively dispersed appearance in the nucleus, and occupies most of the nuclear region in Figure 27.10.
- Some regions of chromatin are very densely packed with fibers, displaying a condition comparable to that of the chromosome at mitosis. This material is called **heterochromatin**. It passes through the cell cycle with relatively little change in its degree of

Figure 27.9

The sister chromatids of a mitotic pair each consist of a fiber (~30 nm in diameter) compactly folded into the chromosome. Photograph kindly provided by E. J. DuPraw.

Figure 27.10

A thin section through a nucleus stained with Feulgen shows heterochromatin as compact regions clustered near the nucleolus and nuclear membrane. Photograph kindly provided by Edmund Puvion.



condensation. It forms a series of discrete clumps in Figure 27.10, but often the various heterochromatic regions aggregate into a densely staining **chromocenter**.

The same fibers run continuously between euchromatin and heterochromatin, which implies that these states represent different degrees of condensation of the genetic material. In the same way, euchromatic regions exist in different states of condensation during interphase and during mitosis. Thus the genetic material is organized in a manner that permits alternative states to be maintained side by side in chromatin, and allows cyclical changes to occur in the packaging of euchromatin between interphase and division.

The structural condition of the genetic material is correlated with its transcriptional activity: chromatin is not expressed in the more condensed state. Mitotic chromosomes provide an extreme case; they are transcriptionally inert, as cells virtually cease transcription during the process of division. Interphase cells contain two classes of heterochromatin, each containing a different type of sequence; and in neither type is the DNA transcribed:

- **Constitutive heterochromatin** consists of particular regions that are not expressed. They include short repeated sequences of DNA (see Chapter 26), and may play a structural role in the chromosome. Often these sequences are concentrated in characteristic regions (see below).

- **Facultative heterochromatin** takes the form of entire chromosomes that are inactive in one cell lineage, although they can be expressed in other lineages. The example *par excellence* is the mammalian X chromosome, one copy of which (selected at random) is entirely inactive in a given female. (This compensates for the presence of two X chromosomes, compared with the one present in males.) The inactive X chromosome is perpetuated in a heterochromatic state, while the active X chromosome is part of the euchromatin. Here it is possible to see a correlation between transcriptional activity and structural organization when the *identical DNA sequences* are involved in both states.

Condensation of the genetic material is thus associated with (perhaps is responsible for)

inactivity. Note, however, that the reverse is not true. Active genes are contained within euchromatin; but only a small minority of the sequences in euchromatin are transcribed at any time. Thus location in euchromatin is *necessary* for gene expression, but is not *sufficient* for it. We may wonder whether the gross changes seen between euchromatin and heterochromatin are mimicked in a lesser manner by changes in the structure of euchromatin, to give transcribed regions a less condensed structure than that of nontranscribed regions.

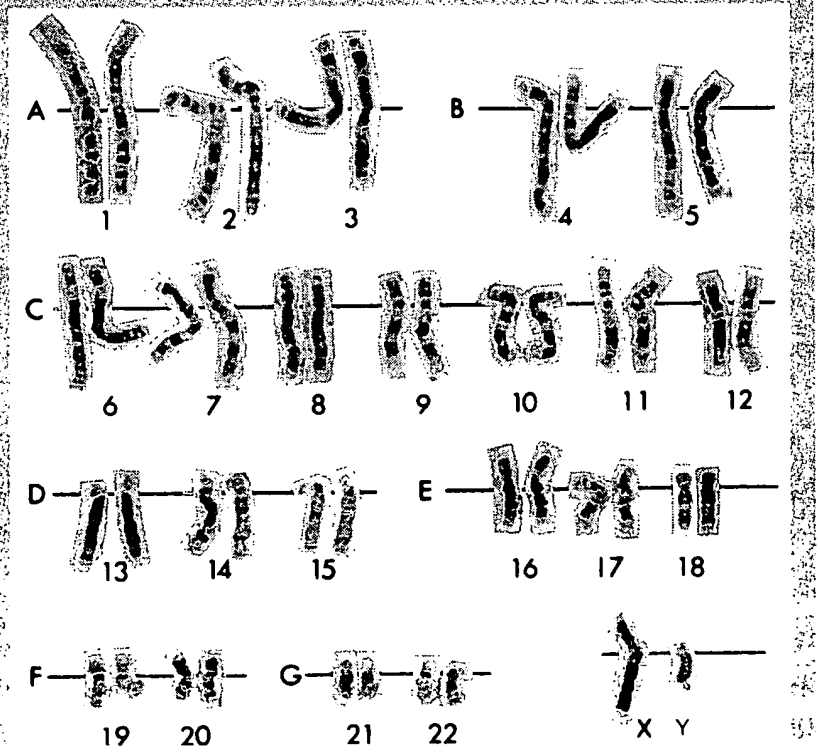
Because of the diffuse state of chromatin, we cannot directly determine the specificity of its organization. But we can ask whether the structure of the chromosome is ordered. Do particular sequences always lie at particular sites, or is the folding of the fiber into the overall structure a more random event?

At the level of the chromosome, each member of the complement has a different and reproducible ultrastructure. When subjected to certain treatments and then stained with the chemical dye Giemsa, chromosomes generate a series of **G-bands**. An example of the human set is presented in **Figure 27.11**.

Until the development of this technique, chromosomes could be distinguished only by their overall size and the relative location of the centromere (see later). Now each chromosome can

Figure 27.11

G-banding generates a characteristic lateral series of bands in each member of the chromosome set. Photograph kindly provided by Lisa Shaffer.



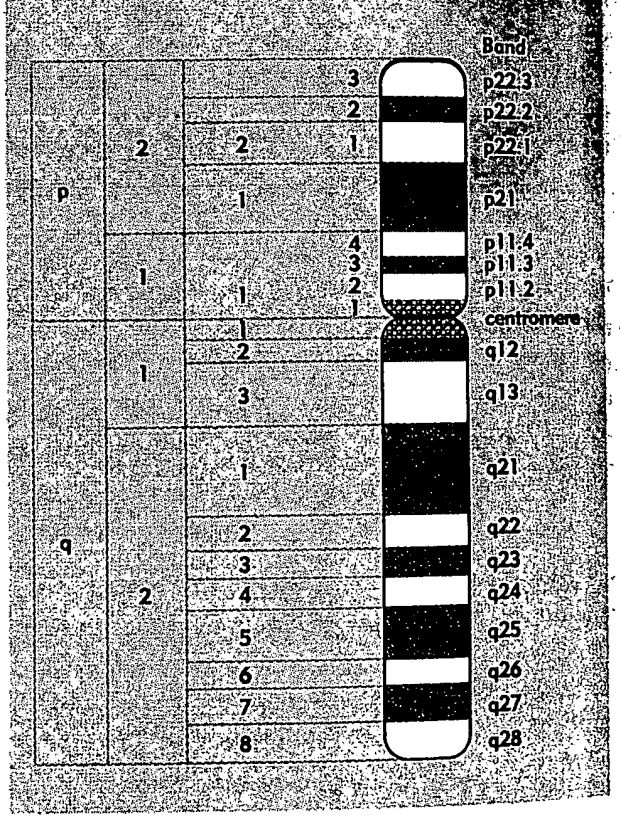
be identified by its characteristic banding pattern. This pattern is reproducible enough to allow translocations from one chromosome to another to be identified by comparison with the original diploid set. Figure 27.12 shows a diagram of the bands of the human X chromosome.

The banding technique is of enormous practical use, but the mechanism of banding remains a mystery. All that is certain is that the dye stains untreated chromosomes more or less uniformly. So the generation of bands depends on a variety of treatments that change the response of the chromosome (presumably by extracting the component that binds the stain from the nonbanded regions). But the variety of effective treatments is so great that no common cause yet has been discerned. These results imply the existence of a definite long-range structure, but its basis is unknown.

Each chromosome contains a single, very long duplex of DNA. This explains why chromosome replication is semiconservative like the individual DNA molecule (see Figure 4.15); this would not necessarily be the case if a chromosome carried many independent molecules of DNA. The single duplex of DNA is folded into the 30 nm fiber, which runs continuously throughout the chromosome. Thus in accounting for interphase chromatin and mitotic chromosome structure, we have to explain the packaging of a single, exceedingly long molecule of DNA into a form in which it can be transcribed and replicated, and can become cyclically more and less compressed.

Figure 27.12

The human X chromosome can be divided into distinct regions by its banding pattern. The short arm is *p* and the long arm is *q*; each arm is divided into larger regions that are further subdivided. This map shows a low resolution structure; at higher resolution, some bands are further subdivided into smaller bands and interbands, e.g. p21 is divided into p21.1, p21.2, and p21.3. A high resolution map is shown in Figure 6.11.



The extended state of lampbrush chromosomes

It would be extremely useful to visualize gene expression in its natural state, to see what structural changes are associated with transcription. But the nature of the material restricts such analysis to some unusual circumstances.

The compression of DNA in chromatin, coupled with the difficulty of identifying particular genes within it, makes it impossible to visualize the

Figure 27.13

A lampbrush chromosome is a meiotic bivalent in which the two pairs of sister chromatids are held together at chiasmata (indicated by arrows). Photograph kindly provided by Joe Gall.

transcription of individual active genes. (However, they do display some distinctive biochemical properties that can be analyzed *in vitro*, as described in Chapter 28.)

Mitotic chromosomes are inert in gene expression, and, in any case, are so compact as to preclude the identification of individual loci. The distinct regions that are rendered discernible by the G-banding technique, as in the example of Figure 27.11, each contain ~10^7 bp of DNA, which could include many hundreds of genes.

Lateral differentiation of structure is evident in many chromosomes when they first appear for meiosis. At this stage, the chromosomes resemble a series of beads on a string. The beads are densely staining granules, properly known as **chromomeres**. However, usually there is little gene expression at meiosis, and it is not practical to use this material to identify the activities of individual genes.

Gene expression can be visualized directly in certain unusual situations, in which the chromosomes are found in a highly extended form that allows individual loci (or groups of loci) to be distinguished. One such situation is presented by **lampbrush chromosomes**, which have been best characterized in certain amphibians.

Lampbrush chromosomes are formed during an unusually extended meiosis, which can last up to several months! During this period, the chromosomes are held in a stretched-out form in which they can be visualized in the light microscope. Later during meiosis, the chromosomes revert to their usual compact size. So the extended state essentially proffers an unfolded version of the normal condition of the chromosome.

The lampbrush chromosomes are meiotic bivalents, each consisting of two pairs of sister chromatids. **Figure 27.13** shows an example in which the sister chromatid pairs have mostly separated so that they are held together only by chiasmata (the sites of crossing-over). Each sister chromatid pair forms a series of ellipsoidal chromomeres, ~1–2 μm in diameter, which are connected by a very fine thread. This thread contains the two sister duplexes of DNA and runs continuously along the chromosome, through the chromomeres.

The lengths of the individual lampbrush chromosomes in the newt *Notophthalmus*

viridescens range from 400 to 800 μm, compared with the range of 15–20 μm seen later in meiosis. So the lampbrush chromosomes are ~30 times less tightly packed. The total length of the entire lampbrush chromosome set is 5–6 mm, organized into about 5000 chromomeres.

The lampbrush chromosomes take their name from the lateral loops that extrude from the chromomeres at certain positions. (These resemble a lampbrush, an extinct object.) The loops extend in pairs, one from each sister chromatid. The loops are continuous with the axial thread, which suggests that they represent chromosomal material extruded from its more compact organization in the chromomere.

The loops are surrounded by a matrix of ribonucleoproteins. These contain nascent RNA chains. Often a transcription unit can be defined by the increase in the length of the RNP moving around the loop. An example is shown in **Figure 27.14**.

So the loop is an extruded segment of DNA that is being actively transcribed. In some cases, loops corresponding to particular genes have been identified. Then the structure of the transcribed gene, and the nature of the product, can be scrutinized *in situ*.

Figure 27.14

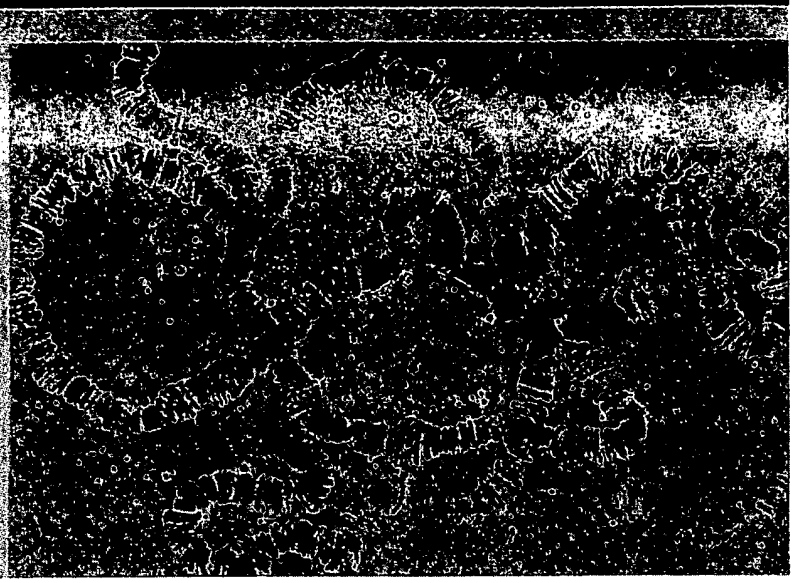
A lampbrush chromosome loop is surrounded by a matrix of ribonucleoprotein. Photograph kindly provided by Oscar Miller.

Transcription disrupts the structure of polytene chromosomes

The interphase nuclei of some tissues of the larvae of Dipteran flies contain chromosomes that are greatly enlarged relative to their usual condition. They possess both increased diameter and greater length. **Figure 27.15** shows an example of a chromosome set from the salivary gland of *D. melanogaster*. They are called **polytene chromosomes**.

Each chromosome consists of a visible series of **bands** (more properly, but rarely, described

Figure 27.15



The polytene chromosomes of *D. melanogaster* form an alternating series of bands and interbands. Photograph kindly provided by Jose Bonner.

chromomeres). The bands range in size from the largest with a breadth of ~0.5 μm to the smallest of ~0.05 μm. (The smallest can be distinguished only under the electron microscope.) The bands contain most of the mass of DNA and stain intensely with appropriate reagents. The regions between them stain more lightly and are called **interbands**. There are ~5000 bands in the *D. melanogaster* set.

The centromeres of all four chromosomes of *D. melanogaster* aggregate to form a chromocenter that consists largely of heterochromatin (in the male it includes the entire Y chromosome). Allowing for this, ~75% of the haploid DNA set is organized into the band-interband alternation.

The length of the chromosome set is ~2000 μm; 75% of the DNA is 1.3×10^8 bp, which would extend for ~40,000 μm, so the average packing ratio is ~20. This demonstrates vividly the extension of the genetic material relative to the usual states of interphase chromatin or mitotic chromosomes.

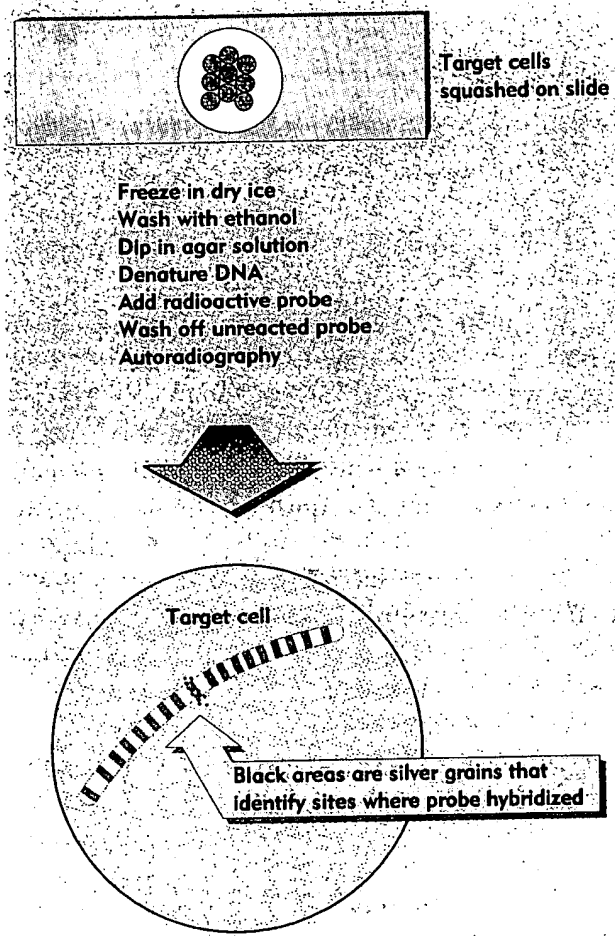
What is the structure of these giant chromosomes? Each is produced by the successive replications of a synapsed diploid pair. The replicas do not separate, but remain attached to each other in their extended state. At the start of the process, each synapsed pair has a DNA content of 2C (where C represents the DNA content of the individual chromosome). Then this doubles up to 9 times, at its maximum giving a content of 1024C. The number of doublings is different in the various tissues of the *D. melanogaster* larva.

Each chromosome can be visualized as a large number of parallel fibers running longitudinally, tightly condensed in the bands, less condensed in the interbands. Probably each fiber represents a single (C) haploid chromosome. This gives rise to the name 'polyteny'. The degree of polyteny is the number of haploid chromosomes contained in the giant chromosome.

The banding pattern is characteristic for each strain of *Drosophila*. The constant number and linear arrangement of the bands was first

Figure 27.16

Individual bands containing particular genes can be identified by *in situ* hybridization.



noted in the 1930s, when it was realized that they form a **cytological map** of the chromosomes. Rearrangements—such as deletions, inversions, or duplications—result in alterations of the order of bands.

The linear array of bands can be equated with the linear array of genes. Thus genetic rearrangements, as seen in a linkage map, can be correlated with structural rearrangements of the cytological map. Ultimately, a particular mutation can be located in a particular band. Since the total number of genes in *D. melanogaster* appears to exceed the number of bands, there are probably multiple genes in most or all bands (see Chapter 24).

The positions of particular genes on the cytological map can be determined directly by the technique of ***in situ*** or **cytological hybridization**. The protocol is summarized in **Figure 27.16**. A radioactive probe representing a gene (most often a labeled cDNA clone derived from the mRNA) is hybridized with the denatured DNA of the polytene chromosomes *in situ*. Autoradiography identifies the position or positions of the corresponding genes by the superimposition of grains at a particular band or bands. An example is shown in **Figure 27.17**. With this type of technique at hand, it is possible to determine directly the band within which a particular sequence lies.

One of the intriguing features of the polytene chromosomes is that active sites can be visualized. Some of the bands pass transiently through an expanded or **puffed** state, in which chromosomal material is extruded from the axis. An example of some very large puffs (called Balbiani rings) is shown in **Figure 27.18**.

What is the nature of the puff? It consists of a region in which the chromosome fibers unwind from their usual state of packing in the band. The fibers remain continuous with those in the chromosome axis. Puffs usually emanate from single bands, although when they are very large, as typified by the Balbiani rings, the swelling may be so extensive as to obscure the underlying array of bands.

The pattern of puffs is related to gene expression. During larval development, puffs appear and regress in a definite, tissue-specific pattern. A characteristic pattern of puffs is found in each tissue at any given time. Puffs are induced by the hormone ecdysone that controls *Drosophila* development. Some puffs are induced directly by the hormone; others are induced indirectly by the products of earlier puffs.

The puffs are *sites where RNA is being synthesized*. The accepted view of puffing has been that expansion of the band is a consequence of the need to relax its structure in order to synthesize

Figure 27.17

A magnified view of bands 87A and 87C shows their hybridization *in situ* with labeled RNA extracted from heat-shocked cells. Photograph kindly provided by Jose Bonner.

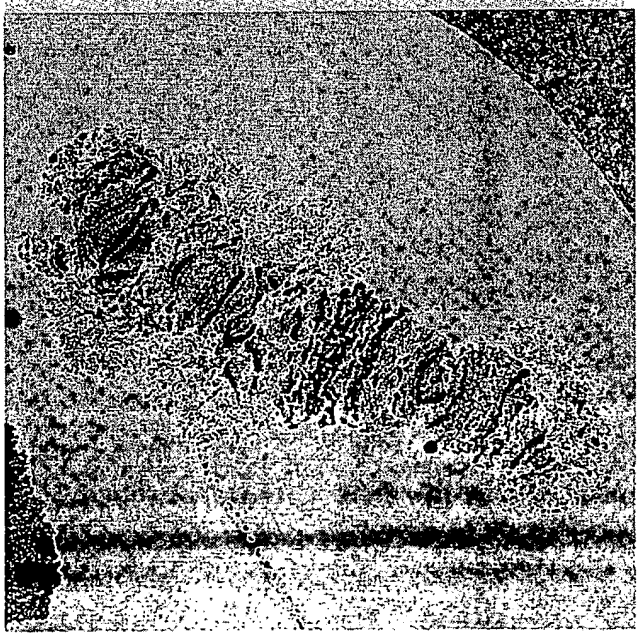
RNA. Puffing has therefore been viewed as a consequence of transcription. A puff can be generated by a single active gene.

The sites of puffing differ from ordinary bands in accumulating additional proteins. Characterization of these proteins at present is only rather primitive. We know that they include RNA polymerase II and other proteins associated with the act of transcription. We should like to analyze the entire set of proteins that accumulate at puffs, in particular to characterize those that are a cause rather than a consequence of the puffing. Then it should be possible to determine the nature of the molecular events responsible for the expansion of material.

The features displayed by lampbrush and polytene chromosomes suggest a general conclusion. In order to be transcribed, the genetic material is dispersed from its usual more tightly packed state. The question to keep in mind is whether this dispersion at the gross level of the chromosome mimics the events that occur at the molecular level within the mass of ordinary interphase euchromatin.

Figure 27.18

Chromosome IV of the insect *C. tentans* has three Balbiani rings in the salivary gland. Photograph kindly provided by Bertil Daneholt.



The eukaryotic chromosome as a segregation device

During mitosis, the sister chromatids move to opposite poles of the cell (as illustrated in Figure 2.21). Their movement depends on the attachment of the chromosome to microtubules, which are connected at their other end to the poles. (The microtubules comprise a cellular filamentous system, reorganized at mitosis so that they connect the chromosomes to the poles of the cell.) The sites in the two regions where microtubule ends are organized—in the vicinity of the centrioles at the poles and at the chromosomes—are called MTOCs (microtubule organizing centers).

The region of the chromosome that is responsible for its segregation at mitosis and meiosis is called the **centromere**. It is associated with two important features:

- It contains the site at which the sister chromatids are held together prior to the separation of the individual chromosomes. This shows as a constricted region connecting all four chromosome arms, as in the photograph of Figure 27.9, which shows the sister chromatids at the metaphase stage of mitosis.
- The term 'centromere' historically has been used in both the functional and structural sense to describe the feature of the chromosome responsible for its movement. The centromere is pulled toward the pole during mitosis, and the attached chromosome is dragged along behind, as it were. The chromosome therefore provides a device for attaching a large number of genes to the apparatus for division.

The centromere is essential for segregation, as shown by the behavior of chromosomes that have been broken. A single break generates one piece that retains the centromere, and another, an **acentric fragment**, that lacks it. The acentric fragment does not become attached to the mitotic spindle; and as a result it fails to be included in either of the daughter nuclei.

(When chromosome movement relies on discrete centromeres, there can be *only* one centromere per chromosome. When translocations generate chromosomes with more than one centromere, aberrant structures form at mitosis, since the two centromeres on the *same* sister chromatid can be pulled toward different poles, breaking the chromosome. However, in some species the centromeres are 'diffuse', which creates a different situation. Only discrete centromeres have been analyzed at the molecular level.)

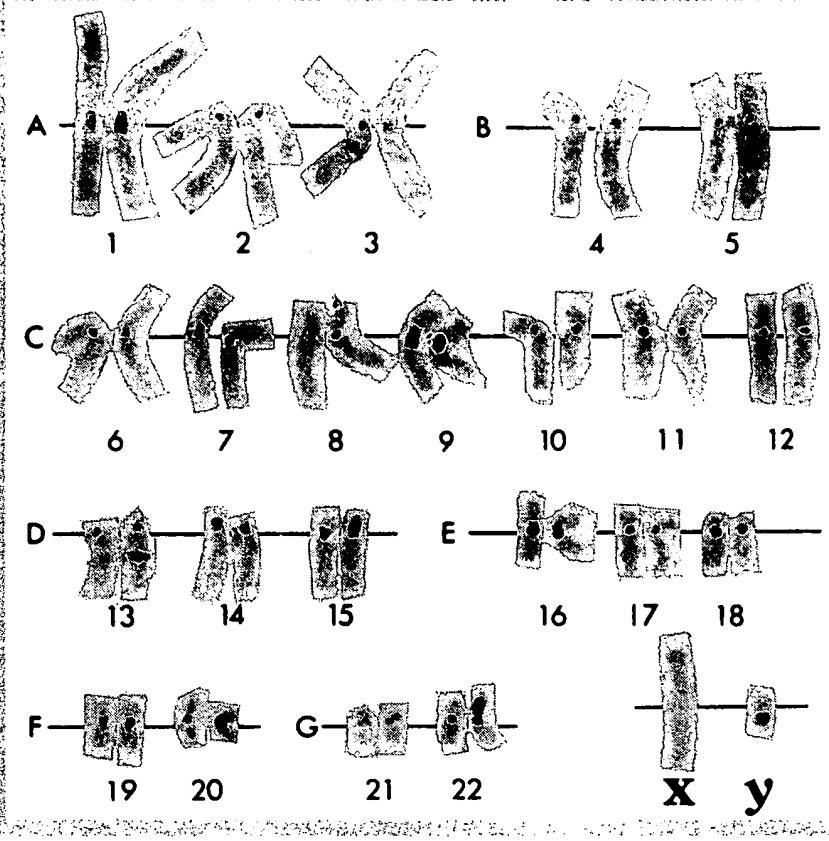
The regions flanking the centromere often are rich in satellite DNA sequences and contain a considerable amount of constitutive heterochromatin. Because the entire chromosome is condensed, centromeric heterochromatin is not immediately evident in mitotic chromosomes. However, it can be visualized by a technique called **C-banding**. In the example of **Figure 27.19**, all the centromeres show as darkly staining regions. Although it is common, constitutive heterochromatin cannot be identified around *every* known centromere, which suggests that it is unlikely to be essential for the division mechanism.

What is the feature of the centromere that is responsible for segregation? Within the centromeric region, a darkly staining fibrous object of diameter or length ~400 nm can be seen. This **kinetochore** appears to be directly attached to the microtubules. Usually it is assumed that a specific sequence of DNA in some way defines the site at which the kinetochore should be established, but so far we have made no progress toward characterizing the molecular location and organization of this structure. The kinetochore provides the MTOC on a chromosome.

If a centromeric sequence of DNA is responsible for segregation, any molecule of DNA possessing this sequence should move properly at cell division, while any DNA lacking it will fail to segregate. This prediction has been used to isolate centromeric DNA in the yeast, *S. cerevisiae*. Yeast

Figure 27.19

C-banding generates intense staining at the centromeres of all chromosomes. Photograph kindly provided by Lisa Shaffer.



chromosomes do not display visible kinetochores comparable to those of higher eukaryotes, but otherwise divide at mitosis and segregate at meiosis by the same mechanisms.

Genetic engineering has produced plasmids of yeast that are replicated like chromosomal sequences (see Chapter 18). However, they are unstable at mitosis and meiosis, disappearing from a majority of the cells because they segregate erratically. Fragments of chromosomal DNA have been isolated by virtue of their ability to confer mitotic stability on these plasmids. Their chromosomal derivations can be identified when they contain genetic markers known to map near a centromere.

A *CEN* fragment is defined by its ability to confer stability upon such a plasmid. By reducing the sizes of the fragments that are incorporated into the plasmid, the minimum region necessary for mitotic centromeric function can be identified. Deletions and other changes can be made to investigate the features involved in centromeric function.

Another way to use the availability of the centromeric sequences is to modify them *in vitro* and then reintroduce them into the yeast cell, where they replace the corresponding centromere on the chromosome. This allows the sequences required for *CEN* function to be defined directly in the context of the chromosome.

A *CEN* fragment derived from one chromosome can replace the centromere of another chromosome with no apparent consequence. This result suggests that centromeres are inter-changeable. *They are used simply to attach the chromosome to the spindle, and play no role in distinguishing one chromosome from another.*

The sequences required for centromeric function fall within a stretch of ~120 bp, in which three types of sequence element may be

distinguished, as summarized in **Figure 27.20**:

- CDE-I is a sequence of 9 bp that is conserved with minor variations at the left boundary of all centromeres.
- CDE-II is a >90% A•T-rich sequence of 80–90 bp found in all centromeres; its function could depend on its length rather than exact sequence. Its constitution is reminiscent of some short tandemly repeated (satellite) DNAs in higher eukaryotes (see Chapter 26). Its base composition may cause some characteristic distortions of the DNA double helical structure.
- CDE-III is an 11 bp sequence highly conserved at the right boundary of all centromeres. Sequences on either side of the element are less well conserved, and may also be needed for centromeric function. (CDE-III could be longer than 11 bp if it turns out that the flanking sequences are essential.)

Mutations in CDE-I or CDE-II reduce but do not inactivate centromere function, but point mutations in the central CCG of CDE-III completely inactivate the centromere. Can we identify proteins that are necessary for the function of CEN sequences? Deletion of the gene coding for a protein (called CBF-I) that binds specifically to CDE-I increases the frequency of mitotic chromosome loss from 10^{-5} to 10^{-4}. (An acentric chromosome is lost at a frequency of 10^{-1}.)

A 240,000 dalton complex of three proteins, CBF-IIIA,B,C, binds to CDE-III, but cannot bind a point mutant that lacks centromeric function. Mutations in the components of the genes coding [illegible] block chromosome movement at mitosis [illegible] protein complex has a microtubule-based motor activity—it is able to move itself, and presumably attached objects such as chromosomes, along microtubules. Comparable proteins with sequences related to known motor activities have been found at eukaryotic centromeres. Taken together, these observations suggest that a protein complex with motor activity may connect the centromeric region of a chromosome to microtubules and contribute to movement on the mitotic spindle. The discovery of the CBF-III complex may be the prelude to characterizing the connection between the centromere and the apparatus for chromosome segregation.

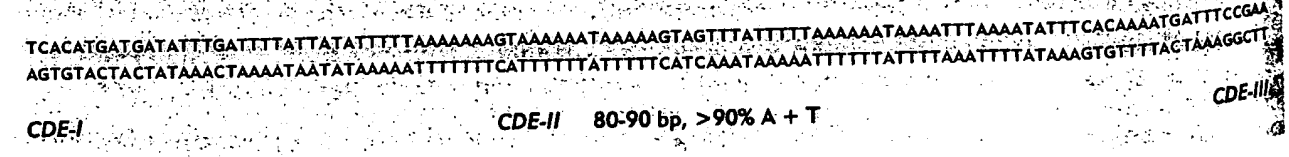
Attempts to characterize functional centromeres from the yeast *S. pombe* have been less successful. They cannot be isolated by ability to confer stability on plasmids. However, *S. pombe* has only 3 chromosomes, and the region containing each centromere has been identified by deleting most of the sequences of each chromosome to create a stable minichromosome. This approach locates the centromeres within regions of 40–100 kb that consist largely or entirely of repetitious DNA. It is not clear how much of each of these rather long regions is required for chromosome segregation at mitosis and meiosis.

The significance of the difference between the short centromeric regions in *S. cerevisiae* and the long regions in *S. pombe* is not clear. The common feature is that the DNA consists of noncoding sequences that are repetitive.

The primary motif comprising the constitutive

Figure 27.20

Three conserved regions can be identified by the sequence homologies between yeast *CEN* elements.



Heterochromatin of primate centromeres is the α satellite DNA, which consists of tandem arrays of a 170 bp repeating unit. There is significant variation between individual repeats, although those at any centromere tend to be better related to one another than to members of the family in other locations. It is clear that the sequences required for centromeric function reside within the blocks of a satellite DNA, but it is not clear whether the α satellite sequences themselves provide this function, or whether other sequences are embedded within the α satellite arrays.

Specific chromatin structures are found at centromeres, and we discuss examples in Chapter 28.

Chromosome ends are special

Another essential feature in all chromosomes is the **telomere**. In some way that we do not yet understand, this 'seals' the end. We know that the telomere must be a special structure, because chromosome ends generated by breakage are 'sticky' and tend to react with other chromosomes, whereas natural ends are stable.

We can apply two criteria in identifying a telomeric sequence:

- It must lie at the end of a chromosome (or, at least, at the end of an authentic linear DNA molecule).
- It must confer stability on a linear molecule.

Several telomeric sequences have been obtained from linear DNA molecules present in the genomes of lower eukaryotes. The same type of sequence is found in plants and man, so the construction of the telomere seems to follow a universal principle. Each telomere consists of a long series of short, tandemly repeated sequences. **Table 27.2** lists the repeating units that have been identified at the ends of the linear DNA molecules. All can be written in the general form $C_n(A/T)_m$, where $n>1$ and m is 1–4.

Within the telomeric region is a specific array of discontinuities, taking the form of single-strand breaks whose structure prevents them from being sealed by the ligase enzyme that normally acts

Table 27.2

Telomeres have a common type of short tandem repeat. The repeating unit gives the sequence of one strand, in the direction from the telomere toward the centromere.

Type of Organism	Species	Source of DNA	Repeating unit (5'-3')
Holotrichous ciliates	*Tetrahymena, Paramecium*	Macronucleus	CCCCAA
Hypotrichous ciliates	*Stylonchia, Oxytricha, Euplotes*	Macronucleus	CCCCAAAA
Flagellates	*Trypanosoma, Leishmania*	Minichromosome	CCCTA
Slime molds	*Physarum, Dictyostelium*	rDNA	CCCTA
Yeast	*Saccharomyces*	Chromosome	$C_{2-3}A(CA)_{1-3}$
Plant	*Arabidopsis*	Chromosome	C_3TA_3
Man	*Homo sapiens*	Chromosome	C_3TA_2

upon nicks in one DNA strand. The very terminal bases are blocked in some way—they may be organized in a hairpin—so that they are not recognized by nucleases.

The problem of finding a system that offers an assay for function again has been brought to the molecular level by using yeast. All the plasmids that survive in yeast (by virtue of possessing *ARS* and *CEN* elements) are circular DNA molecules. Linear plasmids are unstable (because they are degraded). Could an authentic telomeric DNA sequence confer stability on a linear plasmid?

Fragments from yeast DNA that prove to be located at chromosome ends can be identified by such an assay. And a region from the end of a known natural linear DNA molecule—the extrachromosomal rDNA of *Tetrahymena*—is able to render a yeast plasmid stable in linear form. The nicks in the telomeric sequence are perpetuated at the same sites in yeast, a remarkable interspecies conservation.

Some indications about how a telomere functions are given by some unusual properties of the ends of linear DNA molecules. In a trypanosome population, the ends are variable in length. When an individual cell clone is followed, the telomere grows longer by 7–10 bp (1–2 repeats) per generation. Even more revealing is the fate of ciliate telomeres introduced into yeast. After replication in yeast, *yeast telomeric repeats are added onto the ends of the Tetrahymena repeats.*

Addition of telomeric repeats to the end of the chromosome in every replication cycle could solve the problem of replicating linear DNA molecules discussed in Chapter 19. The addition of repeats by *de novo* synthesis would counteract the loss of repeats resulting from failure to replicate up to the end of the chromosome. Extension and shortening would be in dynamic equilibrium.

The overall length of the telomere is under genetic control; different strains of yeast have different but characteristic telomeric lengths. Some mechanism must prevent the ends from growing too long, possibly by removing some of the repeats. Mutation of an essential yeast gene causes the telomeres to grow steadily longer; the function of the wild-type gene could be to limit telomere extension.

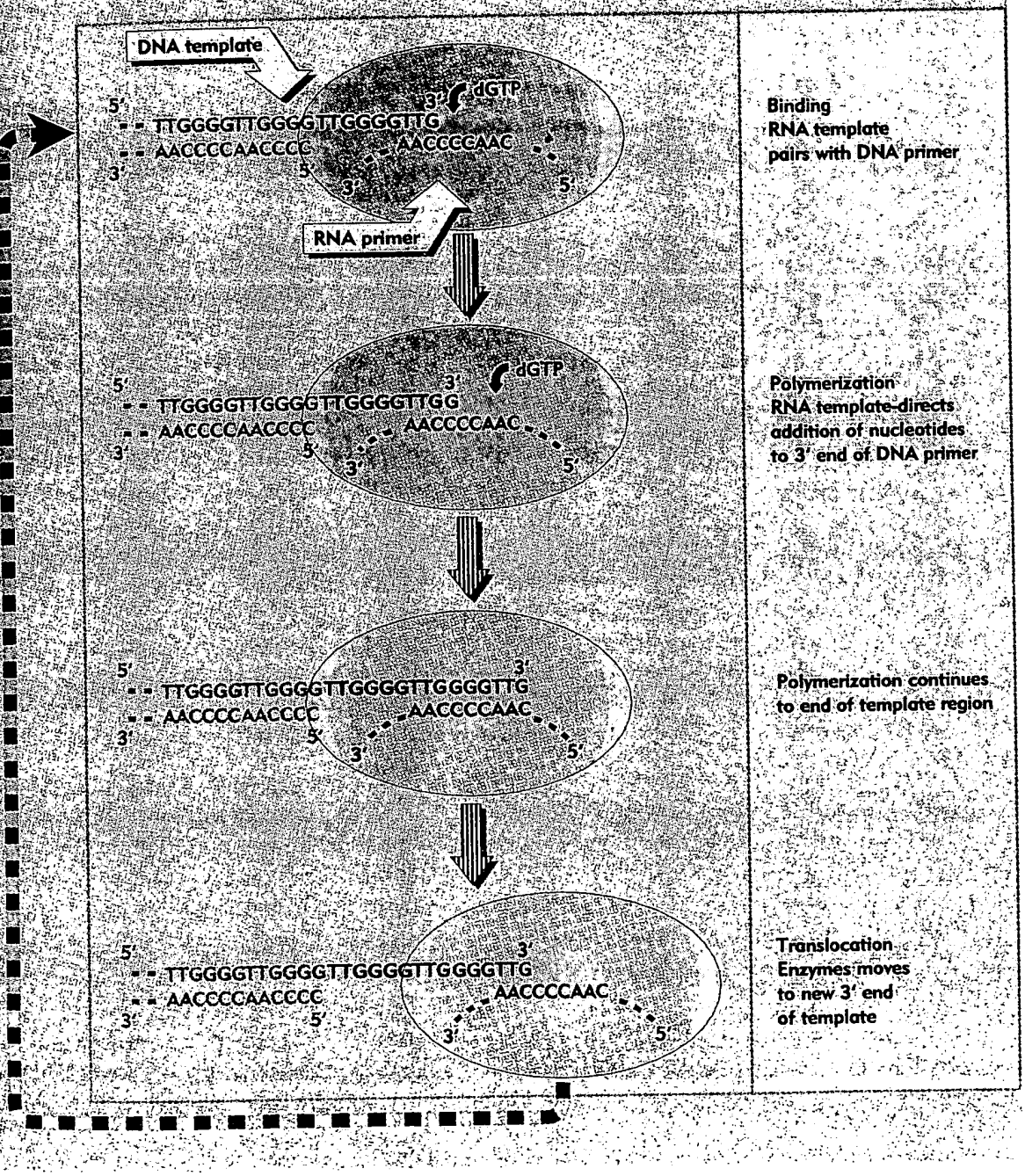
If telomeres are continually being lengthened (and shortened), their exact sequence may be irrelevant. All that is required is for the present end to be recognized as a suitable substrate for addition. This explains how the ciliate telomere functions in yeast. However, we do not yet understand how the telomeric sequence confers resistance of chromosome ends to damage.

How are the telomeric repeats synthesized? Extracts of *Tetrahymena* contain an enzyme, called **telomerase**, that uses the 3′-OH of the G+T telomeric strand as a primer for synthesis of tandem TTGGGG repeats. Only dGTP and dTTP are needed for the activity. The telomerase is a large ribonucleoprotein. It contains a short RNA component, 159 bases long in *Tetrahymena*, 192 bases long in *Euplotes*. Each RNA includes a sequence of 15–22 bases that is identical to two repeats of the C-rich repeating sequence given in Table 27.2. This RNA provides the template for synthesizing the G-rich repeating sequence, to which it is complementary. Bases are added individually, in the correct sequence, as depicted in **Figure 27.21**. The enzyme progresses discontinuously: the template RNA is positioned on the DNA primer, several nucleotides are added to the primer, and then the enzyme translocates to begin again. The telomerase is a specialized example of a reverse transcriptase, an enzyme that synthesizes a DNA sequence using an RNA template (see Chapter 35). The protein component provides the catalytic activity of reverse transcriptase, and is (presumably) confined to acting upon the RNA template provided by the nucleic acid component.

The structure of the telomere is organized as represented in Figure 27.21, with a single-stranded extension of the G-T-rich strand, usually for 14–16 bases. But isolated telomeric fragments do not

Figure 27.21

Telomerase positions itself by base pairing between the RNA template and the protruding single-stranded DNA primer. It adds G and T bases one at a time to the primer, as directed by the template. The cycle starts again when 1 repeating unit has been added.



behave as though they contain single-stranded DNA; instead they show aberrant electrophoretic mobility and other properties. Two models for the structure of the end are depicted in Figure 27.22.

An early model suggested that a duplex hairpin could form if the extended G-T strand folds back on itself by means of unusual base pair interactions. As few as two repeating units could seal the end of the DNA.

A later model proposed the existence of a 'quartet' of G residues, formed by an association of one G from each repeating unit. In the example in the figure, the second G of each of four successive T_2G_4 units forms a member of the quartet. The rest of the repeating unit is looped out. The association between the G residues requires that two of them change the orientation of the base with regard to the sugar (from the usual *anti* to the unusual *syn* configuration). Since each repeating unit has more than one G, more than one quartet could be formed if other G residues associate, in which case quartets might be stacked upon one another in a helical manner.

We do not know how the complementary (C-A-rich) strand of the telomere is assembled, but we may speculate that it could be synthesized by using the 3′-OH of a terminal G-T hairpin as a primer for DNA synthesis.

What feature of the centromere is responsible for the stability of the chromosome end? Telomere-binding proteins recognize the protruding G-rich strand and protect the DNA at the terminus *in vitro*. They may provide the 'cap' that protects the telomere *in vivo*. Other proteins may bind specifically to the regions adjacent to the repeating units, their role remains to be defined.

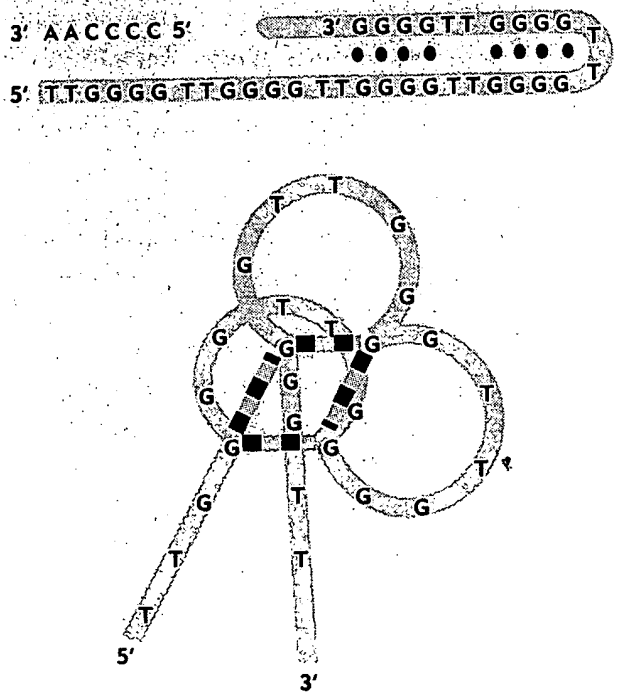
The minimum features required for existence as a chromosome are:

- Telomeres to ensure survival.
- A centromere to support segregation.
- An origin to initiate replication (see Chapter 18).

All of these elements have been put together to construct a yeast artificial chromosome (YAC). We have discussed the use of such chromosomes for perpetuating foreign sequences in Chapter 21. It turns out that the synthetic chromosome is stable only if it is longer than 20–50 kb. We do not know the basis for this effect, but the ability to construct a synthetic chromosome offers the potential to investigate the nature of the segregation device in a controlled environment.

Figure 27.22

The unusual behavior of telomeric fractions may be explained by G-G interactions. In the upper model a duplex hairpin is formed by G-G pairing. In the lower model, a G quartet is formed when 1 G is contributed by each of 4 repeating units.



Summary

The genetic material of all organisms and viruses takes the form of tightly packaged nucleoprotein. Some virus genomes are inserted into preformed virions, while others assemble a protein coat around the nucleic acid. The bacterial genome forms a dense nucleoid, with about 20% protein by mass, but details of the interaction of the proteins with DNA are not known. The DNA is organized into ~100 domains that maintain independent supercoiling, with a density of unrestrained supercoils corresponding to ~1 per 100-200 bp. Interphase chromatin and metaphase chromosomes both appear to be organized into large loops. Each loop may be an independently supercoiled domain. The bases of the loops are connected to a metaphase scaffold or to the nuclear matrix by specific DNA sites.

Transcriptionally active sequences reside within the euchromatin that comprises the majority of interphase chromatin. The regions of heterochromatin are packaged ~5–10× more compactly, and are transcriptionally inert. All chromatin becomes densely packaged during cell division, when the individual chromosomes can be distinguished. The existence of a reproducible ultrastructure in chromosomes is indicated by the production of G-bands by treatment with Giemsa stain. The bands are very large regions, ~10^7 bp, that can be used to map chromosomal translocations or other large changes in structure.

Lampbrush chromosomes of amphibians and polytene chromosomes of insects have unusually extended structures, with packing ratios <100. Polytene chromosomes of *D. melanogaster* are divided into ~5000 bands, varying in size by an order of magnitude, with an average of ~25 kb. Genetic analysis suggests that each band contains 1 essential locus, but molecular analysis suggests that there are several genes within a band. Transcriptionally active regions can be seen to exist in an even more unfolded ('puffed') structure, in which material is extruded from the axis of the chromosome. This may resemble the changes that occur on a smaller scale when a sequence in euchromatin is transcribed.

The centromeric region contains the kinetochore, which is responsible for attaching a chromosome to the mitotic spindle. The centromere often is surrounded by heterochromatin. Centromeric sequences have been identified only in yeast, where they consist of short conserved elements and a long A•T-rich region. Proteins that bind to these sequences have been identified.

Telomeres make the ends of chromosomes stable. Almost all known telomeres consist of multiple repeats in which one strand has the general sequence $C_n(A/T)_m$, where $n > 1$ and $m = 1–4$. The other strand, $G_n(T/A)_m$, has a single protruding end that provides a template for addition of individual bases in defined order. The enzyme telomere transferase is a ribonucleoprotein, whose RNA component provides the template for synthesizing the G-rich strand.

Further reading

Reviews

Packaging of phage DNA has been reviewed by **Black** (*Ann. Rev. Microbiol.* **43**, 267–292, 1989).

The concept of the bacterial nucleoid has been reviewed by **Brock** (*Microbiol. Rev.* **52**, 397–411, 1988).

Proteins potentially involved in bacterial genome structure have been reviewed by **Drlica and Rouviere-Yaniv** (*Microbiol. Rev.* **51**, 301–319, 1987).

Centromeres and telomeres were reviewed by **Blackburn and Szostak** (*Ann. Rev. Biochem.* **53**, 163–194, 1984) and by **Clarke and Carbon** (*Ann. Rev. Genet.* **19**, 29–56, 1985). Centromeres were reviewed by **Schulman and Bloom** (*Ann. Rev. Cell Biol.* **7**, 311–336, 1991). Telomeres have been brought up to date by **Zakian** (*Ann. Rev. Genet.* **23**, 579–604, 1989) and by **Blackburn** (*Nature* **350**, 569–573, 1991). Telomerase was reviewed by **Blackburn** (*Ann. Rev. Biochem.* **61**, 113–129, 1992).

Discoveries

The structure of the centromere was revealed by **Bloom and Carbon** (*Cell* **29**, 305–317, 1982).

The functional elements needed by a chromosome were put together to make a synthetic chromosome by **Murray and Szostak** (*Nature* **305**, 189–193, 1983). Telomerase was discovered by **Greider and Blackburn** (*Cell* **51**, 887–898,1987) and its catalytic activity characterized by **Shippen-Lentz and Blackburn** (*Science* **247**, 546–552, 1990). The unusual structure of the G-T-rich tail was recognized by **Henderson *et al.*** (*Cell* **51**, 899–908, 1987), and a G quartet model was proposed by **Williamson, Raghuraman, and Cech** (*Cell* **59**, 871–880, 1989).

1: NM_003355. Reports Homo sapiens unco...[gi:13259540]

Comment Features Sequence

```
LOCUS       NM_003355               1646 bp    mRNA    linear   PRI 16-APR-2006
DEFINITION  Homo sapiens uncoupling protein 2 (mitochondrial, proton carrier)
            (UCP2), nuclear gene encoding mitochondrial protein, mRNA.
ACCESSION   NM_003355
VERSION     NM_003355.2  GI:13259540
KEYWORDS    .
SOURCE      Homo sapiens (human)
  ORGANISM  Homo sapiens
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            Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini;
            Catarrhini; Hominidae; Homo.
REFERENCE   1  (bases 1 to 1646)
  AUTHORS   Oberkofler,H., Klein,K., Felder,T.K., Krempler,F. and Patsch,W.
  TITLE     Role of peroxisome proliferator-activated receptor-gamma
            coactivator-1alpha in the transcriptional regulation of the human
            uncoupling protein 2 gene in INS-1E cells
  JOURNAL   Endocrinology 147 (2), 966-976 (2006)
   PUBMED   16282353
  REMARK    GeneRIF: The principal mechanisms regulating UCP2 gene expression
            are similar in rats and humans, being consistent with a role for
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REFERENCE   2  (bases 1 to 1646)
  AUTHORS   Rudofsky,G. Jr., Schroedter,A., Schlotterer,A., Voron'ko,O.E.,
            Schlimme,M., Tafel,J., Isermann,B.H., Humpert,P.M., Morcos,M.,
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  TITLE     Functional polymorphisms of UCP2 and UCP3 are associated with a
            reduced prevalence of diabetic neuropathy in patients with type 1
            diabetes
  JOURNAL   Diabetes Care 29 (1), 89-94 (2006)
   PUBMED   16373902
  REMARK    GeneRIF: G-866A polymorphism in the UCP2 gene is associated with a
            reduced risk of diabetic neuropathy in type 1 diabetes.
REFERENCE   3  (bases 1 to 1646)
  AUTHORS   Kovacs,P., Ma,L., Hanson,R.L., Franks,P., Stumvoll,M., Bogardus,C.
            and Baier,L.J.
  TITLE     Genetic variation in UCP2 (uncoupling protein-2) is associated with
            energy metabolism in Pima Indians
  JOURNAL   Diabetologia 48 (11), 2292-2295 (2005)
   PUBMED   16167150
  REMARK    GeneRIF: variation in UCP2 may play a role in energy metabolism,
            but this gene does not contribute significantly to the aetiology of
            type 2 diabetes and/or obesity in Pima Indians.
REFERENCE   4  (bases 1 to 1646)
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AUTHORS Vogler,S., Goedde,R., Miterski,B., Gold,R., Kroner,A., Koczan,D., Zettl,U.K., Rieckmann,P., Epplen,J.T. and Ibrahim,S.M.
TITLE Association of a common polymorphism in the promoter of UCP2 with susceptibility to multiple sclerosis
JOURNAL J. Mol. Med. 83 (10), 806-811 (2005)
PUBMED 16021520
REMARK GeneRIF: UCP2 promoter polymorphism may contribute to multiple sclerosis susceptibility by regulating the level of UCP2 protein in the central nervous and/or the immune systems.

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TITLE Linkage exclusion analysis of two candidate regions on chromosomes 7 and 11: leptin and UCP2/UCP3 are not QTLs for obesity in US Caucasians
JOURNAL Biochem. Biophys. Res. Commun. 332 (2), 602-608 (2005)
PUBMED 15910756
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AUTHORS Lee,K.U., Lee,I.K., Han,J., Song,D.K., Kim,Y.M., Song,H.S., Kim,H.S., Lee,W.J., Koh,E.H., Song,K.H., Han,S.M., Kim,M.S., Park,I.S. and Park,J.Y.
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JOURNAL Circ. Res. 96 (11), 1200-1207 (2005)
PUBMED 15905464
REMARK GeneRIF: UCP2 functions as a physiologic regulator of ROS generation in endothelial cells.

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AUTHORS Park,J.Y., Park,K.G., Kim,H.J., Kang,H.G., Ahn,J.D., Kim,H.S., Kim,Y.M., Son,S.M., Kim,I.J., Kim,Y.K., Kim,C.D., Lee,K.U. and Lee,I.K.
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JOURNAL Diabetologia 48 (5), 1022-1028 (2005)
PUBMED 15827742
REMARK GeneRIF: UCP-2 can modify atherosclerotic processes inhuman vascular muscle smooth cells in response to high glucose and Angiotensin II.

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AUTHORS Gniuli,D., Rosa,G., Manco,M., Scarfone,A., Vega,N., Greco,A.V., Castagneto,M., Vidal,H. and Mingrone,G.
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JOURNAL Obes. Res. 13 (3), 567-573 (2005)
PUBMED 15833942
REMARK GeneRIF: reduction of free fatty acids(FFAs) induced by bilio-pancreatic diversion acts in inhibiting FFA transportation to the mitochondria uncoupling protein 2 (UCP-2), contributing to the decreased lipid oxidation inside the adipose tissue

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AUTHORS Oberkofler,H., Iglseder,B., Klein,K., Unger,J., Haltmayer,M., Krempler,F., Paulweber,B. and Patsch,W.
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JOURNAL Arterioscler. Thromb. Vasc. Biol. 25 (3), 604-610 (2005)
PUBMED 15604415
REMARK GeneRIF: Our results suggest a role of UCP2 in atherogenesis

REFERENCE 10 (bases 1 to 1646)
AUTHORS Fridell,Y.W., Sanchez-Blanco,A., Silvia,B.A. and Helfand,S.L.
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JOURNAL Cell Metab 1 (2), 145-152 (2005)
PUBMED 16054055
REMARK GeneRIF: neuronal-specific expression of hUCP2 in adult flies decreases cellular oxidative damage and is sufficient to extend life span

REFERENCE 11 (bases 1 to 1646)
AUTHORS Bulotta,A., Ludovico,O., Coco,A., Di Paola,R., Quattrone,A., Carella,M., Pellegrini,F., Prudente,S. and Trischitta,V.
TITLE The common -866G/A polymorphism in the promoter region of the UCP-2 gene is associated with reduced risk of type 2 diabetes in Caucasians from Italy
JOURNAL J. Clin. Endocrinol. Metab. 90 (2), 1176-1180 (2005)
PUBMED 15562023
REMARK GeneRIF: Single nucleotid polymorphism is associated with type 2 diabetes risk in Italy.

REFERENCE 12 (bases 1 to 1646)
AUTHORS Andrews,Z.B., Horvath,B., Barnstable,C.J., Elsworth,J., Yang,L., Beal,M.F., Roth,R.H., Matthews,R.T. and Horvath,T.L.
TITLE Uncoupling protein-2 is critical for nigral dopamine cell survival in a mouse model of Parkinson's disease
JOURNAL J. Neurosci. 25 (1), 184-191 (2005)
PUBMED 15634780
REMARK GeneRIF: The present results expose the critical importance of UCP2 in normal nigral dopamine cell metabolism.

REFERENCE 13 (bases 1 to 1646)
AUTHORS Jaburek,M., Miyamoto,S., Di Mascio,P., Garlid,K.D. and Jezek,P.
TITLE Hydroperoxy fatty acid cycling mediated by mitochondrial uncoupling protein UCP2
JOURNAL J. Biol. Chem. 279 (51), 53097-53102 (2004)
PUBMED 15475368
REMARK GeneRIF: UCP2 has a role in hydroperoxy fatty acid cycling

REFERENCE 14 (bases 1 to 1646)
AUTHORS Ando,T., Kodama,N., Ishikawa,T., Naruo,T., Tachikawa,N., Nozaki,T., Okabe,K., Takeuchi,K., Masuda,A., Kawamura,N. and Komaki,G.
TITLE Uncoupling protein-2/uncoupling protein-3 gene polymorphism is not associated with anorexia nervosa
JOURNAL Psychiatr. Genet. 14 (4), 215-218 (2004)
PUBMED 15564896
REMARK GeneRIF: The hypothesis that differences in the UCP-2 genes influence the susceptibility to anorexia nervossa was not supported.

REFERENCE 15 (bases 1 to 1646)
AUTHORS Yamaguchi,H., Jelokhani-Niaraki,M. and Kodama,H.
TITLE Second transmembrane domain of human uncoupling protein 2 is essential for its anion channel formation
JOURNAL FEBS Lett. 577 (1-2), 299-304 (2004)
PUBMED 15527803
REMARK GeneRIF: anion channel structure of UCP2 protein is oligomeric and the second transmembrane domain is essential for the voltage-dependence of this anion channel

REFERENCE 16 (bases 1 to 1646)
AUTHORS Horimoto,M., Resnick,M.B., Konkin,T.A., Routhier,J., Wands,J.R. and Baffy,G.
TITLE Expression of uncoupling protein-2 in human colon cancer
JOURNAL Clin. Cancer Res. 10 (18 PT 1), 6203-6207 (2004)
PUBMED 15448008

REMARK GeneRIF: UCP2 may be part of a novel adaptive response by which oxidative stress is modulated in colon cancer

REFERENCE 17 (bases 1 to 1646)
AUTHORS D'Adamo,M., Perego,L., Cardellini,M., Marini,M.A., Frontoni,S., Andreozzi,F., Sciacqua,A., Lauro,D., Sbraccia,P., Federici,M., Paganelli,M., Pontiroli,A.E., Lauro,R., Perticone,F., Folli,F. and Sesti,G.
TITLE The -866A/A genotype in the promoter of the human uncoupling protein 2 gene is associated with insulin resistance and increased risk of type 2 diabetes
JOURNAL Diabetes 53 (7), 1905-1910 (2004)
PUBMED 15220218
REMARK GeneRIF: The -866A/A genotype was associated with diabetes in women, but not in men. The -866A/A genotype of the UCP2 gene may contribute to diabetes susceptibility by affecting insulin sensitivity.

REFERENCE 18 (bases 1 to 1646)
AUTHORS Ryu,J.W., Hong,K.H., Maeng,J.H., Kim,J.B., Ko,J., Park,J.Y., Lee,K.U., Hong,M.K., Park,S.W., Kim,Y.H. and Han,K.H.
TITLE Overexpression of uncoupling protein 2 in THP1 monocytes inhibits beta2 integrin-mediated firm adhesion and transendothelial migration
JOURNAL Arterioscler. Thromb. Vasc. Biol. 24 (5), 864-870 (2004)
PUBMED 15016641
REMARK GeneRIF: Mitochondrial UCP2 in circulating monocytes may prevent excessive accumulation of monocytes/macrophages in the arterial wall, thereby reducing atherosclerotic plaque formation.

REFERENCE 19 (bases 1 to 1646)
AUTHORS Lindholm,E., Klannemark,M., Agardh,E., Groop,L. and Agardh,C.D.
TITLE Putative role of polymorphisms in UCP1-3 genes for diabetic nephropathy
JOURNAL J. Diabetes Complicat. 18 (2), 103-107 (2004)
PUBMED 15120704
REMARK GeneRIF: No association between the different polymorphisms and diabetic nephropathy.

REFERENCE 20 (bases 1 to 1646)
AUTHORS Cho,Y.M., Ritchie,M.D., Moore,J.H., Park,J.Y., Lee,K.U., Shin,H.D., Lee,H.K. and Park,K.S.
TITLE Multifactor-dimensionality reduction shows a two-locus interaction associated with Type 2 diabetes mellitus
JOURNAL Diabetologia 47 (3), 549-554 (2004)
PUBMED 14730379
REMARK GeneRIF: In type 2 diabetes there is a significant gene to gene interaction between the Ala55Val polymorphism in the uncoupling protein 2 gene (UCP2) and the 161C>T polymorphism in the exon 6 of ppargamma.

REFERENCE 21 (bases 1 to 1646)
AUTHORS Sasahara,M., Nishi,M., Kawashima,H., Ueda,K., Sakagashira,S., Furuta,H., Matsumoto,E., Hanabusa,T., Sasaki,H. and Nanjo,K.
TITLE Uncoupling protein 2 promoter polymorphism -866G/A affects its expression in beta-cells and modulates clinical profiles of Japanese type 2 diabetic patients
JOURNAL Diabetes 53 (2), 482-485 (2004)
PUBMED 14747301
REMARK GeneRIF: UCP2 promoter polymorphism -866G/A does not affect obesity in Japanese type 2 diabetic patients but affects its transcription in beta-cells.

REFERENCE 22 (bases 1 to 1646)
AUTHORS Ji,Q., Ikegami,H., Fujisawa,T., Kawabata,Y., Ono,M., Nishino,M., Ohishi,M., Katsuya,T., Rakugi,H. and Ogihara,T.

TITLE A common polymorphism of uncoupling protein 2 gene is associated with hypertension
JOURNAL J. Hypertens. 22 (1), 97-102 (2004)
PUBMED 15106800
REMARK GeneRIF: Polymorphism of uncoupling protein 2 gene is associated with hypertension, and suggests the possibility of uncoupling protein 2 gene as a target molecule for studies on the etiology and treatment of hypertension.

REFERENCE 23 (bases 1 to 1646)
AUTHORS Le Fur,S., Le Stunff,C., Dos Santos,C. and Bougneres,P.
TITLE The common -866 G/A polymorphism in the promoter of uncoupling protein 2 is associated with increased carbohydrate and decreased lipid oxidation in juvenile obesity
JOURNAL Diabetes 53 (1), 235-239 (2004)
PUBMED 14693721
REMARK GeneRIF: Juvenile obese subjects who are homozygous for the A variant have an increased ratio (3.6 +/- 1.2) of calories derived from carbohydrates to those from lipids.

REFERENCE 24 (bases 1 to 1646)
AUTHORS Wang,H., Chu,W.S., Lu,T., Hasstedt,S.J., Kern,P.A. and Elbein,S.C.
TITLE Uncoupling protein-2 polymorphisms in type 2 diabetes, obesity, and insulin secretion
JOURNAL Am. J. Physiol. Endocrinol. Metab. 286 (1), E1-E7 (2004)
PUBMED 12915397
REMARK GeneRIF: Uncoupling protein-2 variants show significant effects on insulin secretion in interaction with family-specific factors. However, the associated allele and the effects on gene expression are opposite to those reported previously.

REFERENCE 25 (bases 1 to 1646)
AUTHORS Paradis,E., Clavel,S., Bouillaud,F., Ricquier,D. and Richard,D.
TITLE Uncoupling protein 2: a novel player in neuroprotection
JOURNAL Trends Mol Med 9 (12), 522-525 (2003)
PUBMED 14659466
REMARK Review article
GeneRIF: Uncoupling protein 2 has a role in neuroprotection [review]

REFERENCE 26 (bases 1 to 1646)
AUTHORS Dalgaard,L.T., Andersen,G., Larsen,L.H., Sorensen,T.I., Andersen,T., Drivsholm,T., Borch-Johnsen,K., Fleckner,J., Hansen,T., Din,N. and Pedersen,O.
TITLE Mutational analysis of the UCP2 core promoter and relationships of variants with obesity
JOURNAL Obes. Res. 11 (11), 1420-1427 (2003)
PUBMED 14627764
REMARK GeneRIF: variation of the uncoupling protein 2 promoter is not associated with obesity or obesity-related intermediary phenotypes in Danish subjects

REFERENCE 27 (bases 1 to 1646)
AUTHORS Diano,S., Matthews,R.T., Patrylo,P., Yang,L., Beal,M.F., Barnstable,C.J. and Horvath,T.L.
TITLE Uncoupling protein 2 prevents neuronal death including that occurring during seizures: a mechanism for preconditioning
JOURNAL Endocrinology 144 (11), 5014-5021 (2003)
PUBMED 12960023
REMARK GeneRIF: Mitochondrial UCPs precondition neurons by dissociating cellular energy production from that of free radicals to withstand the harmful effects of cellular stress occurring in a variety of neurodegenerative disorders, including epilepsy.

REFERENCE 28 (bases 1 to 1646)
AUTHORS Maestrini,S., Podesta,F., Di Blasio,A.M., Savia,G., Brunani,A.,

Tagliaferri,A., Mencarelli,M., Chiodini,I. and Liuzzi,A.
TITLE Lack of association between UCP2 gene polymorphisms and obesity phenotype in Italian Caucasians
JOURNAL J. Endocrinol. Invest. 26 (10), 985-990 (2003)
PUBMED 14759071
REMARK GeneRIF: UCP2 allelic variants may not have a direct role in the pathogenesis and development of obesity.
REFERENCE 29 (bases 1 to 1646)
AUTHORS Echtay,K.S., Esteves,T.C., Pakay,J.L., Jekabsons,M.B., Lambert,A.J., Portero-Otin,M., Pamplona,R., Vidal-Puig,A.J., Wang,S., Roebuck,S.J. and Brand,M.D.
TITLE A signalling role for 4-hydroxy-2-nonenal in regulation of mitochondrial uncoupling
JOURNAL EMBO J. 22 (16), 4103-4110 (2003)
PUBMED 12912909
REFERENCE 30 (bases 1 to 1646)
AUTHORS Teshima,Y., Akao,M., Jones,S.P. and Marban,E.
TITLE Uncoupling protein-2 overexpression inhibits mitochondrial death pathway in cardiomyocytes
JOURNAL Circ. Res. 93 (3), 192-200 (2003)
PUBMED 12855674
REMARK GeneRIF: Overexpression of human UCP2 attenuates reactive oxygen species generation and prevents mitochondrial Ca2+ overload in cultured neonatal rat cardiomyocytes revealing a novel mechanism of cardioprotection
REFERENCE 31 (bases 1 to 1646)
AUTHORS Mattiasson,G., Shamloo,M., Gido,G., Mathi,K., Tomasevic,G., Yi,S., Warden,C.H., Castilho,R.F., Melcher,T., Gonzalez-Zulueta,M., Nikolich,K. and Wieloch,T.
TITLE Uncoupling protein-2 prevents neuronal death and diminishes brain dysfunction after stroke and brain trauma
JOURNAL Nat. Med. 9 (8), 1062-1068 (2003)
PUBMED 12858170
REMARK GeneRIF: Data suggest that uncoupling protein-2 is an inducible protein that is neuroprotective by activating cellular redox signaling or by inducing mild mitochondrial uncoupling that prevents the release of apoptogenic proteins.
REFERENCE 32 (bases 1 to 1646)
AUTHORS Jaburek,M. and Garlid,K.D.
TITLE Reconstitution of recombinant uncoupling proteins: UCP1, -2, and -3 have similar affinities for ATP and are unaffected by coenzyme Q10
JOURNAL J. Biol. Chem. 278 (28), 25825-25831 (2003)
PUBMED 12734183
REMARK GeneRIF: study of isolation, refolding, transport properties, and regulation of recombinant UCP2
REFERENCE 33 (bases 1 to 1646)
AUTHORS Horvath,T.L., Diano,S. and Barnstable,C.
TITLE Mitochondrial uncoupling protein 2 in the central nervous system: neuromodulator and neuroprotector
JOURNAL Biochem. Pharmacol. 65 (12), 1917-1921 (2003)
PUBMED 12787871
REMARK Review article
GeneRIF: the recent advancements on the role of UCP2 in the brain and portray this uncoupler as an important player in normal neuronal function as well as a key cell death-suppressing device.
REFERENCE 34 (bases 1 to 1646)
AUTHORS Zackova,M., Skobisova,E., Urbankova,E. and Jezek,P.
TITLE Activating omega-6 polyunsaturated fatty acids and inhibitory purine nucleotides are high affinity ligands for novel mitochondrial uncoupling proteins UCP2 and UCP3

JOURNAL J. Biol. Chem. 278 (23), 20761-20769 (2003)
PUBMED 12670931
REMARK GeneRIF: purine nucleotides must be the physiological inhibitors of UCP2-mediated uncoupling in vivo.
REFERENCE 35 (bases 1 to 1646)
AUTHORS Vettor,R., Mingrone,G., Manco,M., Granzotto,M., Milan,G., Scarda,A., Lombardi,A., Greco,A.V. and Federspil,G.
TITLE Reduced expression of uncoupling proteins-2 and -3 in adipose tissue in post-obese patients submitted to biliopancreatic diversion
JOURNAL Eur. J. Endocrinol. 148 (5), 543-550 (2003)
PUBMED 12720538
REMARK GeneRIF: UCPs in adipose tissue may play a role in the reduction in 24-h energy expenditure observed in post-obese individuals.
REFERENCE 36 (bases 1 to 1646)
AUTHORS Sesti,G., Cardellini,M., Marini,M.A., Frontoni,S., D'Adamo,M., Del Guerra,S., Lauro,D., De Nicolais,P., Sbraccia,P., Del Prato,S., Gambardella,S., Federici,M., Marchetti,P. and Lauro,R.
TITLE A common polymorphism in the promoter of UCP2 contributes to the variation in insulin secretion in glucose-tolerant subjects
JOURNAL Diabetes 52 (5), 1280-1283 (2003)
PUBMED 12716765
REMARK GeneRIF: the common -866G/A polymorphism in the UCP2 gene may contribute to the biological variation of insulin secretion
REFERENCE 37 (bases 1 to 1646)
AUTHORS Schauble,N., Geller,F., Siegfried,W., Goldschmidt,H., Remschmidt,H., Hinney,A. and Hebebrand,J.
TITLE No evidence for involvement of the promoter polymorphism -866 G/A of the UCP2 gene in childhood-onset obesity in humans
JOURNAL Exp. Clin. Endocrinol. Diabetes 111 (2), 73-76 (2003)
PUBMED 12746756
REMARK GeneRIF: No evidence for involvement of the promoter polymorphism -866 G/A of the UCP2 gene in childhood-onset obesity in humans
REFERENCE 38 (bases 1 to 1646)
AUTHORS Duarte,N.L., Colagiuri,S., Palu,T., Wang,X.L. and Wilcken,D.E.
TITLE A 45-bp insertion/deletion polymorphism of uncoupling protein 2 in relation to obesity in Tongans
JOURNAL Obes. Res. 11 (4), 512-517 (2003)
PUBMED 12690079
REMARK GeneRIF: there is a unique, near-uniform distribution of the uncoupling protein 2 insertion/deletion polymorphism in Tonga which may be relevant to the prevalence of obesity and type 2 diabetes
REFERENCE 39 (bases 1 to 1646)
AUTHORS Volcik,K.A., Shaw,G.M., Zhu,H., Lammer,E.J. and Finnell,R.H.
TITLE Risk factors for neural tube defects: associations between uncoupling protein 2 polymorphisms and spina bifida
JOURNAL Birth Defects Res. Part A Clin. Mol. Teratol. 67 (3), 158-161 (2003)
PUBMED 12797456
REMARK GeneRIF: These data are the first to suggest that polymorphisms in the UCP2 gene may be genetic risk factors of spina bifida.
REFERENCE 40 (bases 1 to 1646)
AUTHORS Krempler,F., Esterbauer,H., Weitgasser,R., Ebenbichler,C., Patsch,J.R., Miller,K., Xie,M., Linnemayr,V., Oberkofler,H. and Patsch,W.
TITLE A functional polymorphism in the promoter of UCP2 enhances obesity risk but reduces type 2 diabetes risk in obese middle-aged humans
JOURNAL Diabetes 51 (11), 3331-3335 (2002)
PUBMED 12401727
REMARK GeneRIF: A functional polymorphism in the promoter enhances obesity

risk but reduces type 2 diabetes risk in obese middle-aged humans

REFERENCE 41 (bases 1 to 1646)
AUTHORS Jekabsons,M.B., Echtay,K.S. and Brand,M.D.
TITLE Nucleotide binding to human uncoupling protein-2 refolded from bacterial inclusion bodies
JOURNAL Biochem. J. 366 (PT 2), 565-571 (2002)
PUBMED 12030845
REMARK GeneRIF: hypothesis that recombinant human uncoupling protein-2 (UCP2) ectopically expressed in bacterial inclusion bodies binds nucleotides in a manner identical with the nucleotide-inhibited uncoupling that is observed in kidney mitochondria

REFERENCE 42 (bases 1 to 1646)
AUTHORS Harper,M.E., Dent,R., Monemdjou,S., Bezaire,V., Van Wyck,L., Wells,G., Kavaslar,G.N., Gauthier,A., Tesson,F. and McPherson,R.
TITLE Decreased mitochondrial proton leak and reduced expression of uncoupling protein 3 in skeletal muscle of obese diet-resistant women
JOURNAL Diabetes 51 (8), 2459-2466 (2002)
PUBMED 12145158
REMARK GeneRIF: Decreased mitochondrial proton leak and reduced expression in skeletal muscle of obese diet-resistant women

REFERENCE 43 (bases 1 to 1646)
AUTHORS Muzzin,P.
TITLE The uncoupling proteins
JOURNAL Ann. Endocrinol. (Paris) 63 (2 PT 1), 106-110 (2002)
PUBMED 11994670
REMARK Review article
GeneRIF: review

REFERENCE 44 (bases 1 to 1646)
AUTHORS Brown,J.E., Thomas,S., Digby,J.E. and Dunmore,S.J.
TITLE Glucose induces and leptin decreases expression of uncoupling protein-2 mRNA in human islets
JOURNAL FEBS Lett. 513 (2-3), 189-192 (2002)
PUBMED 11904148
REMARK GeneRIF: Glucose induces and leptin decreases expression of uncoupling protein-2 mRNA in human islets.

REFERENCE 45 (bases 1 to 1646)
AUTHORS Saleh,M.C., Wheeler,M.B. and Chan,C.B.
TITLE Uncoupling protein-2: evidence for its function as a metabolic regulator
JOURNAL Diabetologia 45 (2), 174-187 (2002)
PUBMED 11935148
REMARK Review article
GeneRIF: evidence for its function as a metabolic regulator (REVIEW)

REFERENCE 46 (bases 1 to 1646)
AUTHORS Echtay,K.S., Roussel,D., St-Pierre,J., Jekabsons,M.B., Cadenas,S., Stuart,J.A., Harper,J.A., Roebuck,S.J., Morrison,A., Pickering,S., Clapham,J.C. and Brand,M.D.
TITLE Superoxide activates mitochondrial uncoupling proteins
JOURNAL Nature 415 (6867), 96-99 (2002)
PUBMED 11780125

REFERENCE 47 (bases 1 to 1646)
AUTHORS Hu,X., Murphy,F., Karwautz,A., Li,T., Freeman,B., Franklin,D., Giotakis,O., Treasure,J. and Collier,D.A.
TITLE Analysis of microsatellite markers at the UCP2/UCP3 locus on chromosome 11q13 in anorexia nervosa
JOURNAL Mol. Psychiatry 7 (3), 276-277 (2002)
PUBMED 11920154
REMARK GeneRIF: microsatellite markers at the UCP2/UCP3 locus on

chromosome 11q13 in anorexia nervosa
REFERENCE 48 (bases 1 to 1646)
AUTHORS Esterbauer,H., Schneitler,C., Oberkofler,H., Ebenbichler,C., Paulweber,B., Sandhofer,F., Ladurner,G., Hell,E., Strosberg,A.D., Patsch,J.R., Krempler,F. and Patsch,W.
TITLE A common polymorphism in the promoter of UCP2 is associated with decreased risk of obesity in middle-aged humans
JOURNAL Nat. Genet. 28 (2), 178-183 (2001)
PUBMED 11381268
REFERENCE 49 (bases 1 to 1646)
AUTHORS Pierrat,B., Ito,M., Hinz,W., Simonen,M., Erdmann,D., Chiesi,M. and Heim,J.
TITLE Uncoupling proteins 2 and 3 interact with members of the 14.3.3 family
JOURNAL Eur. J. Biochem. 267 (9), 2680-2687 (2000)
PUBMED 10785390
REFERENCE 50 (bases 1 to 1646)
AUTHORS Ricquier,D. and Bouillaud,F.
TITLE The uncoupling protein homologues: UCP1, UCP2, UCP3, StUCP and AtUCP
JOURNAL Biochem. J. 345 PT 2, 161-179 (2000)
PUBMED 10620491
REMARK Review article
REFERENCE 51 (bases 1 to 1646)
AUTHORS Jezek,P. and Urbankova,E.
TITLE Specific sequence of motifs of mitochondrial uncoupling proteins
JOURNAL IUBMB Life 49 (1), 63-70 (2000)
PUBMED 10772343
REFERENCE 52 (bases 1 to 1646)
AUTHORS Pecqueur,C., Cassard-Doulcier,A.M., Raimbault,S., Miroux,B., Fleury,C., Gelly,C., Bouillaud,F. and Ricquier,D.
TITLE Functional organization of the human uncoupling protein-2 gene, and juxtaposition to the uncoupling protein-3 gene
JOURNAL Biochem. Biophys. Res. Commun. 255 (1), 40-46 (1999)
PUBMED 10082652
REFERENCE 53 (bases 1 to 1646)
AUTHORS Argyropoulos,G., Brown,A.M., Peterson,R., Likes,C.E., Watson,D.K. and Garvey,W.T.
TITLE Structure and organization of the human uncoupling protein 2 gene and identification of a common biallelic variant in Caucasian and African-American subjects
JOURNAL Diabetes 47 (4), 685-687 (1998)
PUBMED 9568704
REFERENCE 54 (bases 1 to 1646)
AUTHORS Hodny,Z., Kolarova,P., Rossmeisl,M., Horakova,M., Nibbelink,M., Penicaud,L., Casteilla,L. and Kopecky,J.
TITLE High expression of uncoupling protein 2 in foetal liver
JOURNAL FEBS Lett. 425 (2), 185-190 (1998)
PUBMED 9559644
REFERENCE 55 (bases 1 to 1646)
AUTHORS Suzuki,Y., Yoshitomo-Nakagawa,K., Maruyama,K., Suyama,A. and Sugano,S.
TITLE Construction and characterization of a full length-enriched and a 5'-end-enriched cDNA library
JOURNAL Gene 200 (1-2), 149-156 (1997)
PUBMED 9373149
REFERENCE 56 (bases 1 to 1646)
AUTHORS Vidal-Puig,A., Solanes,G., Grujic,D., Flier,J.S. and Lowell,B.B.
TITLE UCP3: an uncoupling protein homologue expressed preferentially and abundantly in skeletal muscle and brown adipose tissue

JOURNAL Biochem. Biophys. Res. Commun. 235 (1), 79-82 (1997)
PUBMED 9196039
REFERENCE 57 (bases 1 to 1646)
AUTHORS Boss,O., Samec,S., Paoloni-Giacobino,A., Rossier,C., Dulloo,A., Seydoux,J., Muzzin,P. and Giacobino,J.P.
TITLE Uncoupling protein-3: a new member of the mitochondrial carrier family with tissue-specific expression
JOURNAL FEBS Lett. 408 (1), 39-42 (1997)
PUBMED 9180264
REFERENCE 58 (bases 1 to 1646)
AUTHORS Gimeno,R.E., Dembski,M., Weng,X., Deng,N., Shyjan,A.W., Gimeno,C.J., Iris,F., Ellis,S.J., Woolf,E.A. and Tartaglia,L.A.
TITLE Cloning and characterization of an uncoupling protein homolog: a potential molecular mediator of human thermogenesis
JOURNAL Diabetes 46 (5), 900-906 (1997)
PUBMED 9133562
REFERENCE 59 (bases 1 to 1646)
AUTHORS Fleury,C., Neverova,M., Collins,S., Raimbault,S., Champigny,O., Levi-Meyrueis,C., Bouillaud,F., Seldin,M.F., Surwit,R.S., Ricquier,D. and Warden,C.H.
TITLE Uncoupling protein-2: a novel gene linked to obesity and hyperinsulinemia
JOURNAL Nat. Genet. 15 (3), 269-272 (1997)
PUBMED 9054939
REFERENCE 60 (bases 1 to 1646)
AUTHORS Maruyama,K. and Sugano,S.
TITLE Oligo-capping: a simple method to replace the cap structure of eukaryotic mRNAs with oligoribonucleotides
JOURNAL Gene 138 (1-2), 171-174 (1994)
PUBMED 8125298
COMMENT REVIEWED REFSEQ: This record has been curated by NCBI staff. The reference sequence was derived from AF096289.1.
On Mar 9, 2001 this sequence version replaced gi:4507804.

Summary: Mitochondrial uncoupling proteins (UCP) are members of the larger family of mitochondrial anion carrier proteins (MACP). UCPs separate oxidative phosphorylation from ATP synthesis with energy dissipated as heat, also referred to as the mitochondrial proton leak. UCPs facilitate the transfer of anions from the inner to the outer mitochondrial membrane and the return transfer of protons from the outer to the inner mitochondrial membrane. They also reduce the mitochondrial membrane potential in mammalian cells. Tissue specificity occurs for the different UCPs and the exact methods of how UCPs transfer H+/OH- are not known. UCPs contain the three homologous protein domains of MACPs. This gene is expressed in many tissues, with the greatest expression in skeletal muscle. It is thought to play a role in nonshivering thermogenesis, obesity and diabetes. Chromosomal order is 5'-UCP3-UCP2-3'.
COMPLETENESS: full length.

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